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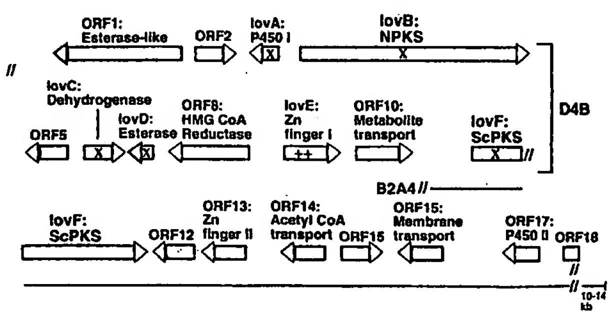
Lovastatin production genes



(57) Abstract

A method of increasing the production of lovastatin or monacolin J in a lovastatin-producing or non-lovastatin-producing organism is disclosed. In one embodiment, the method comprises the steps of transforming an organism with the *A. terreus* D4B segment, wherein the segment is translated and where an increase in lovastatin production occurs.

METHOD OF PRODUCING ANTIHYPERCHOLESTEROLEMIC AGENTS

CROSS-REFERENCES TO RELATED APPLICATION

Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

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BACKGROUND OF THE INVENTION

Cholesterol and other lipids are transported in body fluids by low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Substances that effectuate mechanisms for lowering LDL-cholesterol may serve as effective antihypercholesterolemic agents because LDL levels are positively correlated with the risk of coronary artery disease.

MEVACOR (lovastatin; mevinolin) and ZOCOR (simvastatin) are members of a group of active antihypercholesterolemic agents that function by inhibiting the rate-limiting step in cellular cholesterol biosynthesis, namely the conversion of hydroxymethylglutarylcoenzyme A (HMG-CoA) into mevalonate by HMG-CoA reductase.

The general biosynthetic pathway of a naturally occurring HMG-CoA reductase inhibitor has been outlined by Moore, et al., who showed that the biosynthesis of

mevinolin (lovastatin) by *Aspergillus terreus* ATCC 20542 begins with acetate and proceeds via a polyketide pathway (R.N. Moore, et al., J. Amer. Chem. Soc. 107:3694-3701, 1985). Endo, et al. described similar biosynthetic pathways in *Pencillium citrinum* NRRL 8082 and *Monascus ruber* M-4681 (A.Y. Endo, et al., J. Antibiot. 38:444-448, 1985).

The recent commercial introduction of microbial HMG-CoA reductase inhibitors has fostered a need for high yielding production processes. Methods of improving process yield have included scaling up the process, improving the culture medium and simplifying the isolation.

Previous attempts to increase the biosynthesis of HMG-CoA reductase inhibitors at the level of gene expression have focused on increasing the concentration triol polyketide synthase (TPKS), a multifunctional protein with at least six activities as evidenced by the product of the enzymatic activity (Moore, supra, 1985). TPKS is believed to be the rate-limiting enzymatic activity(ies) in the biosynthesis of the HMG-CoA reductase inhibitor compounds.

U.S. patent 5,744,350 identifies a DNA encoding triol polyketide synthase (TPKS) from *Aspergillus terreus*. "NPKS" is now preferred to TPKS as the acronym for "nonaketide polyketide synthase."

SUMMARY OF THE INVENTION

In one embodiment, the present invention is a method of increasing the production of lovastatin in a lovastatin-producing organism. The method comprises the steps of transforming the organism with a nucleic acid sequence comprising the D4B segment, preferably comprising nucleotides 579 - 33,000 of SEQ ID NO:18 and 1 - 5,349 of SEQ ID NO:19. The nucleic acid sequence is transcribed and translated and an increase in lovastatin production occurs. Preferably, this increase is at least 2-fold.

In a preferred form of the present invention, the lovastatin-producing organism is selected from the group consisting *A. terreus* ATCC 20542 and ATCC 20541.

In another embodiment, the method comprises the step of transforming the organism with the corresponding D4B segment isolated from a non-*A. terreus* lovastatin-producing organism.

In another embodiment, the present invention is a method of increasing the production of lovastatin in a lovastatin-producing organism, comprising the step of transforming the organism with the LovE gene, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

In another embodiment of the present invention, one may increase the production of monacolin J in a non-lovastatin-producing organism comprising the steps of

transforming the organism with a nucleic acid sequence comprising the D4B segment. As a further step, one may additionally transform the organism with an entire LovF gene. If the entire LovF gene is added to the D4B segment, the organism will produce lovastatin.

In another embodiment, the present invention is the lovastatin production gene cluster, preferably SEQ ID NOs:18 and 19, and the individual genes comprising that cluster.

It is an object of the present invention to provide a method for increasing lovastatin and monacolin J production in both lovastatin-producing and non-lovastatin producing organisms.

Other objects, features and advantages of the present invention will become apparent after review of the specification, claims and drawings.

DESCRIPTION OF DRAWINGS

Fig. 1 is a diagram of lovastatin production genes.

Fig. 2 is a schematic diagram of a hypothetical mevinolin/lovastatin biosynthesis pathway.

Fig. 3 is a comparative diagram of statins.

Fig. 4 is a schematic drawing of plasmid pWHM1264/CB24A.

Fig. 5 is a schematic drawing of plasmid pWHM1424.

Fig. 6 is a schematic drawing of plasmid CD4B/pWHM1263.

DESCRIPTION OF THE INVENTION

In General

The Examples below disclose the cloning and sequencing of a cluster of 17 genes from *A. terreus* ATCC 20542, a strain that natively produces lovastatin (See Fig. 1). These genes flank the NPKS gene, which is known to be required for lovastatin production (see, for example, U.S. patent 5,744,350).

The DNA sequence of the cluster has been determined and is disclosed below at SEQ ID NOs:18 and 19. Mutations in four of the genes (P450I/*LovA*, SEQ ID NO:22; dehydrogenase/*LovC*, SEQ ID NO:24; esterase/*LovD*, SEQ ID NO:25; and ScPKS/*LovF*, SEQ ID NO:29) have been isolated and demonstrate that each of these four individual genes is required for lovastatin production. These genes are indicated with an X symbol in Fig. 1 and referred to herein as the "*A. terreus* lovastatin gene cluster."

Another of the genes (Zn Finger I/*LovE*, SEQ ID NO:27) is thought to regulate the transcription of the other genes and causes a notable increase in lovastatin production when reintroduced into *A. terreus* ATCC 20542.

Applicants have used the following convention in naming the genes and proteins of the present invention. The genes and proteins are first named with either an "ORF" or "Lov" prefix and then named either numerically or alphabetically. "Lov" signifies genes shown to be essential for lovastatin production. Applicants have

also included a descriptor name that describes a probable function of the protein. For example, SEQ ID NO:1 is described as the "ORF1/esterase-like protein" because Applicants have compared the amino acid sequence to known esterases.

The portion of the gene cluster between ORF1/esterase-like protein and the mid-region of LovF/SCPKS is referred to as the "D4B segment". The *A. terreus* D4B segment is contained within a plasmid clone deposited as ATCC 98876. As described below, other lovastatin-producing organisms contain an analogous D4B segment comprising analogous genes. The present invention comprises a "D4B segment" isolated from other lovastatin-producing organisms. The arrangment of the genes within the D4B segment may be different in other organisms. We predict that the genes within these other segments will have at least 80% homology, at the nucleic acid level, with the genes disclosed herein. We envision that each of these lovastatin-producing organisms will comprise within their genomes a LovA, LovB, LovC, LovD, LovE and LovF gene.

We have determined that the D4B segment will confer production of monocolin J if the genes are all expressed, as we show below in an example using *A. nidulans*. We envision that adding the LovF gene to the D4B segment genes will result in the production of lovastatin in a non-lovastatin-producing organism.

Table 1, below, summarizes information regarding the different protein and nucleic acid sequences of the present invention. SEQ ID NOs:1-17 are predicted translation products of various members of the gene cluster. SEQ ID NOs:18 and 19 are the entire DNA sequence of the gene cluster. SEQ ID NOs:21-36 are the genomic DNA sequences of the various members of the gene cluster and include the introns. These DNA sequences are reported in the Sequence Listing in the 5' - 3' orientation, although, as Fig. 1 indicates, some of these DNA sequences are in the inverted orientation in the actual cluster.

TABLE 1

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 1	Predicted amino acid sequence of ORF1/Esterase-like protein	Translation of 6 EXONS 6865-6568, 6462-5584, 5520-4822, 4774-3511, 3332-2372, 2301-1813 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 2	Predicted amino acid sequence of ORF2	Translation of 1 EXON 7616-8602 FROM SEQ ID NO:18
SEQ ID NO: 3	Predicted amino acid sequence of LovA/P4501 protein	Translation of 1 EXON 10951-9980 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 4	Predicted amino acid sequence of ORF5	Translation of 1 EXON 22760-21990 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 5	Predicted amino acid sequence of LovC/Dehydrogenase	Translation of 3 EXONS 23158-23717, 23801-23912, 23991-24410 FROM SEQ ID NO:18
SEQ ID NO: 6	Predicted amino acid sequence of LovD/Esterase	Translation of 3 EXONS 26203-26080, 26005-25017, 24938-24810 (reverse complement) FROM SEQ ID NO:18

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 7	Predicted amino acid sequence of ORF8/HMG CoA Reductase	Translation of 5 EXONS 30062-29882, 29803-29745, 29664-27119, 27035-26779, 26722-26559 (reverse complement) FROM SEQ ID NO:18
SEQ ID NO: 8	Predicted amino acid sequence of LovE/Zn Finger I	Translation of 1 EXON 31360-32871 FROM SEQ ID NO:18
SEQ ID NO: 9	Predicted amino acid sequence of ORF10/Metabolite transport	Translation of 8 EXONS 1400-1452, 1619-1695, 1770-1996, 2065-2088, 2154-2225, 2332-2865, 2939-3099, 3180-3560 FROM SEQ ID NO:19
SEQ ID NO: 10	Predicted amino acid sequence of LovF/ScPKS	Translation of 7 EXONS 4430-4627, 4709-4795, 4870-4927, 4985-5318, 5405-5912, 5986-6565, 6631-12464 FROM SEQ ID NO:19
SEQ ID NO: 11	Predicted amino acid sequence of ORF12	Translation of 3 EXONS 13596-13496, 13451-13063, 12968-12709 (reverse complement) FROM SEQ ID NO: 19
SEQ ID NO: 12	Predicted amino acid sequence of ORF13/Zn Finger II	Translation of 5 EXONS 16608-16463, 16376-15572, 15519-15346, 15291-14825, 14767-14131 (reverse complement) FROM SEQ ID NO: 19
SEQ ID NO: 13	Predicted amino acid sequence of ORF14/Acetyl CoA transport protein	Translation of 7 EXONS 19642-19571, 19502-19427, 19352-19227, 19158-19011, 18956-18663, 18587-18438, 18380-18341 (reverse complement) FROM SEQ ID NO:19
SEQ ID NO: 14	Predicted amino acid sequence of ORF15	Translation of 2 EXONS 20332-20574, 20631-21860 FROM SEQ ID NO:19
SEQ ID NO: 15	Predicted amino acid sequence of ORF16/Membrane transport protein	Translation of 5 EXONS 24521-24054, 23996-23936, 23876-23184, 23111-22977, 22924-22818 (reverse complement) FROM SEQ ID NO:19
SEQ ID NO: 16	Predicted amino acid sequence of ORF17/P450II protein	Translation of 3 EXONS 28525-27673, 27606-27284, 27211-26837 (reverse complement) FROM SEQ ID NO:19

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 17	Predicted amino acid sequence of ORF18 (incomplete)	Translation of 2 EXONS 29826-30995. 31054-31328 (incomplete) FROM SEQ ID NO:19
SEQ ID NO: 18	DNA sequence of gene cluster-first 33,000 nucleotides	
SEQ ID NO: 19	DNA sequence of cluster-nucleotides 33,001-64,328 (renumbered 1-31,328)	
SEQ ID NO: 20	DNA sequence of ORF1/Esterase-like gene	Start = 6865 Stop = 1813 SEQ ID NO:18
SEQ ID NO: 21	DNA sequence of ORF2	Start = 7616 Stop = 8602 SEQ ID NO:18
SEQ ID NO: 22	DNA sequence of LovA/P450I gene	Start = 10951 Stop = 9980 SEQ ID NO:18
SEQ ID NO: 23	DNA sequence of ORF5	Start = 22760 Stop = 21990 SEQ ID NO:18
SEQ ID NO: 24	DNA sequence of LovC/Dehydrogenese	Start = 23158 Stop = 24410 SEQ ID NO:18
SEQ ID NO: 25	DNA sequence of LovD/Esterase	Start = 24810 Stop = 26203 SEQ ID NO:18
SEQ ID NO: 26	DNA sequence of ORF8/HMG CoA Reductase	Start = 30062 Stop = 26559 SEQ ID NO:18
SEQ ID NO: 27	DNA sequence of LovE/Zn Finger I	Start = 31360 Stop = 32871 SEQ ID NO:18
SEQ ID NO: 28	DNA sequence of ORF10/Metabolite transport	Start = 1400 Stop = 3560 SEQ ID NO:19
SEQ ID NO: 29	DNA sequence of LovF/ScPKS	Start = 4430 Stop = 12464 SEQ ID NO:19
SEQ ID NO: 30	DNA sequence of ORF12	Start = 13596 Stop = 12709 SEQ ID NO:19

SEQ ID NO.	DESCRIPTION	COMMENTS
SEQ ID NO: 31	DNA sequence of ORF13/Zn Finger II	Start = 16608 Stop = 14131 SEQ ID NO:19
SEQ ID NO: 32	DNA sequence of ORF14/Acetyl CoA transport gene	Start = 19642 Stop = 18341 SEQ ID NO:19
SEQ ID NO: 33	DNA sequence of ORF15	Start = 20332 Stop = 21860 SEQ ID NO:19
SEQ ID NO: 34	DNA sequence of ORF16/Membrane transport protein	Start = 24521 Stop = 22818 SEQ ID NO:19
SEQ ID NO: 35	DNA sequence of ORF17/P450II gene	Start = 28525 Stop = 26837 SEQ ID NO:19
SEQ ID NO: 36	DNA sequence of ORF18 (incomplete)	Start = 29826 to 31328 (incomplete) SEQ ID NO:19

Table 1 also notes the translation start and stop points in the various gene sequences.

The sequence of the NPKS gene is not listed in SEQ ID NOs:21-36. This gene is characterized in U.S. patent 5,744,350. However, SEQ ID NOs:18 and 19 do contain the sequence of the NPKS gene within the context of the entire gene cluster.

To perform many embodiments of the present invention, one will need to recreate various genes or a portion of the gene cluster described herein. Applicants have provided sequence data in the Sequence Listing sufficient to allow one of skill in the art to construct numerous probes suitable to recreate the genes from an *A. terreus* genomic library. Applicants have also described below various methods for isolating *A. terreus* DNA.

Additionally, Applicants have deposited ATCC Accession No. ATCC 98876, which contains clone pWHM1263 (cD4B) and ATCC Accession No. ATCC 98877 which contains clone pWHM1265 (CB2A4). Both plasmids are described in more detail below. Fig. 4 describes clone CB2A4/pWHM1265, and Fig. 6 describes clone CB4B/pWHM1263. Fig. 1 also indicates the boundaries of the D4B and B2A4 clones.

The clones and their inserts may be prepared from the ATCC deposits by methods known to those of skill in the art. The DNA from the clones may be isolated and any gene within the gene cluster may be isolated and utilized.

Increasing the Production of Lovastatin by Lovastatin-producing Fungi and Yeast

In one embodiment, the present invention is a method of increasing the production of lovastatin in a lovastatin-producing fungi and yeast, preferably *A. terreus* ATCC20542 and ATCC20541. Other examples of suitable lovastatin-producing fungi and yeast are listed in Table 2, below.

TABLE 2

Microorganisms other than *A. terreus* reported to produce lovastatin (mevinolin)
Monascus (17 of 124 strains screened) species[1]
M. ruber
M. purpureus
M. pilosus
M. vitreus
M. pubigerus
Penicillium sp.[1]
Hypomyces sp.
Doratomyces sp.
Phoma sp.
Eupenicillium sp.
Gymnoascus sp.
Trichoderma sp.
Pichia labacensis[2]
Candida cariosilognicola
Aspergilus oryzea[3]
Doratomyces stemonitis
Paecilomyces virioti
Penicillium citrinum
Penicillin chrysogenum
Scopulariopsis brevicaulis
Trichoderma viride
1. P. Juzlova, L. Martinkova, V. Kren. Secondary Metabolites of the fungus Monascus: a review. J. Ind. Microbiol. 16:163-170 and references cited therein (1996). 2. N. Gunde-Cimerman, A. Plemenitas and A. Cimerman. A hydroxymethylglutaryl-CoA reductase inhibitor synthesized by yeasts. FEMS Microbiol. Lett. 132:39-43 (1995). 3. A.A. Shindia. Mevinolin production by some fungi. Folio Microbiol. 42:477-480 (1997).

By "increasing the production" we mean that the amount of lovastatin produced is increased by at least 2-fold, preferably by at least 5-fold. The examples below demonstrate two preferred methods for analyzing strains for lovastatin production. In method A, the spore suspension is inoculated into a flask of SEED medium and grown. The resulting seed culture is used to inoculate FM media and grown for six days. In fermentation method

B, one inoculates 50 ml of RPM media and grows this larger culture for 7 days.

Both cultures are extracted, pH adjusted, mixed with ethyl acetate and shaken for two hours. For analysis, 1 ml of the ethyl acetate layer is dried under a nitrogen stream and resuspended in methanol. For TLC analysis, a small amount of the extract is run on C18 reverse phase TLC plates in a solvent system of methanol; 0.1% phosphoric acid. The TLC plates are developed by spraying with phosphomolybdic acid in methanol and heating with a heat gun. The extracts are compared with authentic lovastatin, monacolin J, monacolin L and dihydromonocolon L.

If one wishes HPLC analysis, the examples below describe the use of a Waters Nova-Pak C18 column used with a solvent system of acetonitrile and phosphoric acid. A Waters 996 Photodiode Array Detector will detect the metabolites. Lovastatin was detected at 238 nm.

In one embodiment, one would transform a lovastatin-producing fungi or yeast with the lovE/zinc finger I gene, preferably comprising the nucleotides of SEQ ID NO:27. The examples below predict that this will result in an increase of at least 5-7 fold. Preferably, the increase will be at least 2.0-fold.

One may also transform a lovastatin-producing fungi or yeast with the LovE gene isolated from other lovastatin-producing fungi or yeast. One may obtain this

gene by use of a probe derived from SEQ ID NO:27 by methods known to those of skill in the art.

One may also transform lovastatin-producing fungi and yeast with the D4B segment of the lovastatin production gene cluster (see Fig. 1), preferably as found in ATCC accession number 98876. Alternatively, one may transform lovastatin-producing fungi or yeast with the entire gene cluster, as diagramed in Fig. 1.

We envision that to successfully increase lovastatin production, one may also wish to transform less than the entire gene cluster. Preferably, one may determine what the smallest possible segment is by deleting various portions of the gene cluster and determining whether lovastatin production is continually increased. Similarly, if one begins with the D4B segment, one may delete various portions for the segment and determine whether lovastatin production is continually increased by at least 2-fold.

Modification of the LovB/NPKS gene would produce other HMG CoA inhibitors. For example, Fig. 3 diagrams the relationship between mevastatin, lovastatin, simvastatin and pravastatin. In one example, the methyl transferase domain of the NPKS gene may be replaced with an inactive form to make pravastatin. The HMG-CoA reductase inhibitors within this invention include, but are not limited to, compactin (ML-236B), lovastatin, simvastatin, pravastatin and mevastatin.

In another embodiment of the present invention, one may transform a lovastatin-producing organism with the genes described above and obtain the production of an HMG CoA reductase inhibitor with a structure different from monacolin J, monacolin L or lovastatin. Alterations in the side chain attached to C8 are the most likely possibility but other alterations may occur. These alterations would happen through the native biochemistry of the organism.

If one wishes to express the *A. terreus* genes in yeast, one may wish to consult examples in which others have engineered fungal secondary metabolism genes for expression in yeast. (See for example, J. T. Kealey, et al., Proc. Natl. Acad. Sci. USA 95:505-509 (1998)). The exact approach could be used with the NPKS (LovB) and ScpKS (LovF) genes, and a somewhat simpler approach with the other lovastatin genes in their cDNA forms.

Production of HMG-CoA Reductase Inhibitors by Fungi and Yeast That Do Not Natively Produce Inhibitors.

In another embodiment, the present invention is the production of HMG-CoA reductase inhibitors, such as lovastatin, by fungi and yeast that do not natively produce lovastatin. An example of a suitable fungi or yeast is *A. nidulans* and *S. cerevisiae*, respectively.

For this embodiment one preferably transforms the genes within the D4B segment into the non-inhibitor-producing strain. By this method, one would produce

monacolin J (See Fig. 2) which could be chemically converted to lovastatin by one of skill in the art.

Monacolin J, in its lactone form obtained by treatment with anhydrous acid under dehydrative conditions, is preferably treated with a derivative of (2S)-2-methybutyric acid, in which the carboxyl group has been suitable activated for undergoing esterification, and the resulting lovastatin is isolated by conventional methods. For example, see WO 33538, U.S. patent 4,444,784 and J. Med. Chem. 29:849 (1986). These are citations for synthesis of simvastatin from monacolin J. One would use the same method, but use the (2S)-2-methylbutyrate derivative to make lovastatin.

In another embodiment of the present invention, one would transform the genes within the D4B segment, including an entire LovF/SCPKS gene, into the non-inhibitor-producing organism. By this method, one would produce lovastatin in a non-lovastatin-producing organism.

In another embodiment of the present invention, one may transform a non-lovastatin-producing organism with the genes described above and obtain the production of an HMG CoA reductase inhibitor with a structure different from monacolin J, monacolin L or lovastatin, as described above.

Modification of the LovB/NPKS gene would produce other inhibitors. For example, Fig. 3 diagrams the relationship between mevastatin, lovastatin, simvastatin

and pravastatin. In one example, the methyl transferase domain of the NPKS gene may be replaced with an inactive form to make pravastatin. The HMG-CoA reductase inhibitors within this invention include, but are not limited to, compactin (ML-236B), lovastatin, simvastatin, pravastatin and mevastatin.

Production of Intermediate Materials

In another embodiment, the present invention is a method of isolating intermediate materials in the production of lovastatin and analogs such as mevastatin and simvastatin. For example, the Examples below demonstrate the disruption of the lovastatin projection gene cluster with mutagenized LovC, LovD, LovF, LovA or LovB genes. Disruption of many of these genetic elements of the lovastatin production gene cluster will result in accumulation of intermediate materials. Therefore, to practice this embodiment of the present invention, one would transform a suitable lovastatin-producing host with a mutagenized gene within the D4B segment, as described below.

Many other mutations would be suitable to destroy the function of LovC, LovD, LovF, LovA or LovB. All that is necessary is these genes be disrupted to the extent that they are non-functional.

Production of Lovastatin Analogs

In another embodiment, the present invention provides a method for engineering the production of

lovastatin analogs in such organisms as fungi or yeast, using monacolin J as the starting point.

Isolated DNA Segments

In another embodiment, the present invention is a DNA segment capable of conferring lovastatin or monacolin J production or increase in lovastatin or monacolin J production in yeast or fungi. In a preferred example, this segment is the "D4B segment" that is deposited at ATCC 98876. The nucleotide sequence of this segment is found in residues 579 - 33,000 of SEQ ID NO:18 and residues 1 - 5,349 of SEQ ID NO:19.

In another embodiment, the present invention is the entire *A. terreus* lovastatin gene cluster, as exemplified by SEQ ID NOs:18 and 19 and ATCC deposits 98876 and 98877.

The present invention is also the individual genes that make up the *A. terreus* lovastatin gene cluster. Therefore, the present invention is a nucleic acid segment selected from the group of consisting of SEQ ID NOs:20 - 36. Preferably, the present invention is the coding region found within SEQ ID NOs:20 - 36 and described in Table 1. The present invention is also a mutagenized version of SEQ ID NOs:22, 24, 25 and 29, wherein the gene is mutagenized to be non-functional in terms of lovastatin or monacolin J production.

Organisms with Increased Lovastatin or Monacolin J Production

In another embodiment, the present invention are the organisms described above. These organisms include lovastatin-producing organisms, preferably yeast and fungi, that have been engineered to display at least a 2-fold increase in lovastatin or monacolin J production. The organisms also include non-lovastatin-producing organisms, preferably yeast or fungi, that have been engineered to produce monacolin J or lovastatin.

Antifungal Compounds

Applicants note that lovastatin, monocolin J, monocolin L and dihydromonocolin L all have varying degrees of antifungal activity. Applicants envision that the present invention is also useful for providing antifungal compounds and organisms engineered to express antifungal compounds. Preferably, one would measure the antifungal properties of a compound in the manner of N. Lomovskaya, et al., Microbiology 143:875-883, 1997. Measurement of inhibition of yeast growth can be found in R. Ikeura, et al., J. Antibiotics 41:1148, 1988. The same general methods could be used for all fungi. Both of these references are hereby incorporated by reference.

EXAMPLES

1. General Methods and Procedures

Construction of an *A. terreus* ATCC20542 genomic library.

A. terreus ATCC20542 genomic DNA was partially digested with *Sau*3AI so as to produce an average fragment size of 40 - 50 kb. The partially digested genomic DNA was then separated on a sucrose gradient and the 40 - 50 kb fraction was collected. Cosmid AN26 (Taylor and Borgmann, Fungal Genet. Newsletter 43, 1996) was prepared by digestion with *Cla*I, dephosphorylated with CIP, then digested with *Bam*HI to create the two cosmid arms. Ligation reactions with genomic DNA fragments and cosmid arms were optimized and packaged using Gigapack III XL packaging extract (Stratagene). The packaged cosmid library was infected into *E. coli* JM109 and plated out onto LB agar (Sambrook, et al., Molecular Cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbour Laboratory Press, 1989; other standard methods used can be found here also) with ampicillin (50 μg/ml) plates. After checking for the presence of insert DNA in a selection of clones, 5000 colonies were picked into LB plus 50 μg/ml ampicillin filled microtitre plates and grown overnight at 37°C. The colonies were replica plated onto nylon membranes (Amersham Hybond-N). Glycerol was added at a final concentration of 15% (v/v) to the microtitre plates and these were stored at -70°C.

Isolation of genomic clones containing the lovastatin biosynthesis cluster.

A 2.8 kb *Eco*RI fragment from pTPKS100 containing part of the NPKS gene (Vinci, et al., U.S. Patent No. 5,744,350) was gel-isolated and labelled with digoxigenin using the Genius Kit II (Boehringer Mannheim). This labelled fragment was hybridized (65°C, 5x SSC) with the nylon membranes containing the *A. terreus* genomic library, then washed (65°C, 0.1x SSC). Two positive clones were identified, pWHM1263 (cD4B) and pWHM1264 (cJ3A). Two of these clones, pWHM1263 (cD4B) and pWHM1265 (cB2A4), have been deposited in the ATCC (American Type Culture Collection, 10801 University Boulevard, Menassas, VA 20110) at accession number ATCC 98876 and 98877, respectively, under the terms and conditions of the Budapest Treaty. The presence of the NPKS gene was confirmed initially by restriction digestion and later by DNA sequencing.

Overlapping clones were found by repeating the hybridization process using labelled fragments from both ends of the insert in pWHM1263. This resulted in the isolation of pWHM1265-1270 (cB2A4, cL3E2, cJ3B5, cO2B5, cR3B2, cW3B1) from downstream of the NPKS gene and pWHM1271 (cQ1F1) from upstream of NPKS. All these clones were transformed into *E. coli* strain STBL2 (Stratagene) to help prevent rearrangements.

Fig. 4 is a diagram of the cB2A4/pWHM1265 clone. This clone contains an insert of approximately 43 kb in

AN26 and includes the nucleotide sequence from at least nucleotides 4988 of SEQ ID NO:19 to nucleotide 31,328 of SEQ ID NO:19 and 10 - 14 kb of uncharacterized DNA. Fig. 6 is a schematic diagram of cD4B/pWHM1263. This clone contains a 37,770 bp insert in AN26 and contains nucleotides 579 - 33,000 of SEQ ID NO:18 and nucleotides 1 - 5,349 of SEQ ID NO:19.

Sequencing strategy and analysis.

A series of overlapping subclones (pWHM1272-pWHM1415) were constructed in pSPORT1 (Gibco-BRL) and pGEM3 (Promega). Plasmid DNAs for sequencing were prepared using the QiaPrep spin miniprep kit (Qiagen). Cycle sequencing was carried out using the AmpliTaq FS or BigDye reagents (ABI) and were analyzed using a ABI model 373 or 377 DNA Sequencer. Primer walking was performed by synthesis of 18-22 bp oligonucleotide primers based on the sequenced DNA strand, with the help of the Oligo 4.05 program (National Biosciences, Inc.). Every region of DNA was sequenced at least once on both strands. Direct sequencing of cosmids and PCR products was used to confirm adjoining regions where no overlapping clones existed. DNA sequence analysis and manipulations were performed using SeqMan (DNASTAR) and SeqEd (ABI) software. Assignments of putative ORFS, including putative introns, were performed with the aid of BLAST 2.0 searches (Atschul, et al., Nucl. Acids Res. 25:3389-3402, 1997), and the Genetics Computer Group (GCG) programs (Program Manual for the Wisconsin Package,

Version 8, September 1994, Genetics Computer Group, Madison, WI), version 8.1.

Isolation and characterization of *lovF* (ScPKS, ORF11), *lovD* (EST1, ORF7), *lovC* (DH, ORF6), and *lovA* (P450I, ORF3) mutants.

lovF

To disrupt the polyketide synthase gene, *lovF*, a 1.7 kb *Eco*RI fragment internal to the *lovF* gene was subcloned from pWHM1265 into pSPORT1 to give pWHM1291. The ScPKS fragment was then subcloned from this vector, as an *Acc*65I - *Hin*dIII fragment, into pPLOA (Vinci, et al., U.S. Patent No. 5,744,350) to give pWHM1416. This vector contains the phleomycin (Zeocin, obtained from InVitrogen) resistance gene for selection in *A. terreus*. *A. terreus* ATCC20542 was then transformed to Zeocin resistance with this plasmid as described below. Transformants were screened for lovastatin production as described below (Method A). In one of the transformants, WMH1731, lovastatin production was abolished and a new compound accumulated. This new compound comigrated with monacolin J on TLC and HPLC according to the methods described below. Semi-preparative HPLC was used to partially purify the major product which was then analyzed by HPLC - MS. The same mass and fragmentation pattern as authentic monacolin J was observed. To confirm the disruption of the *lovF* gene, total genomic DNA was prepared from wild-type *A. terreus* ATCC20542 and the WMH1731 mutant strain. The genomic DNA was digested

with *Bam*HI and *Hin*dIII, electrophoresed on an agarose gel and capillary blotted onto a nylon membrane. The membrane was hybridized with the 1.7 kb *Eco*RI fragment from pWHM1416 labelled using the Genius II kit (Boehringer Mannheim) using the conditions described previously. The wild-type strain had hybridizing bands at 4.2 kb for *Bam*HI and 11.5 kb for *Hin*dIII. As predicted, the WMH1731 mutant strain had hybridizing bands at 6.5 kb and 2.2 kb for *Bam*HI and 11 kb and 7.8 kb for *Hin*dIII confirming the homologous integration of a single copy of pWHM1416 at the *lovF* locus.

lovD

To disrupt the putative esterase/carboxypeptidase-like gene, *lovD*, a 4.8 kb *Not*I - *Eco*RI fragment from pWHM1263 was subcloned into pSPORT1 to give pWHM1274. This plasmid was digested with *Hin*dIII and *Bsi*WI and a 1.8 kb fragment was isolated. The plasmid was also digested with *Hin*dIII and *Bam*HI and the 6.6 kb fragment was isolated. pPLOA was digested with *Bam*HI and *Acc*65I and the 2.1 kb fragment containing the phleomycin resistance marker was purified. These three fragments were ligated together and used to transform competent *E. coli* cells. The expected plasmid, pWHM1417, containing the phleomycin resistance gene flanked by the beginning and the end of the *lovD* gene was isolated. This plasmid was linearized by digestion with *Xba*I or *Rsr*II before

being used to transform *A. terreus* ATCC20542 to Zeocin resistance. Transformants were screened for lovastatin production as described below (Method A). In one of the transformants, WMH1732, lovastatin production was abolished and a new compound accumulated. This new compound comigrated with monacolin J on TLC and HPLC according to the methods described below. Semi-preparative HPLC was used to partially purify the major product which was then analyzed by HPLC - MS. The same mass and fragmentation pattern as authentic monacolin J was observed. To confirm the disruption of the *lovD* gene, total genomic DNA was prepared from wild type *A. terreus* ATCC20542 and the WMH1732 mutant strain. The genomic DNA was digested with *ApaI*, run out on an agarose gel and capillary blotted onto a nylon membrane. The membrane was hybridized with the 4.8 kb *NotI* - *EcoRI* fragment from pWHM1274 labelled using the Genius II kit using the conditions described previously. The wild-type strain had hybridizing bands at 9 kb, 8.4 kb and 1.5 kb. As predicted the mutant strain had hybridizing bands at 9 kb, 8 kb, 3 kb and 1.5 kb confirming the homologous integration of a single copy of pWHM1417 at the *lovD* locus.

lovA

To disrupt the cytochrome P450 I gene, *lovA*, an 11 kb *Acc65I* - *EcoRI* fragment from pWHM1263 was subcloned into pGEM3 to give pWHM1272. From this plasmid a 2.1 kb

ApaI - *SnaBI* fragment was purified and ligated to *ApaI* - *EcoRV* digested pPLOA to give p450Phleo (pWHM1418). From this plasmid a 4.2 kb *ApaI* - *NotI* fragment was purified and ligated with a 1.8 kb *EagI* - *KpnI* fragment from pWHM1272 and *ApaI* - *KpnI* digested pGEM7 to give p450Dphleo (pWHM1419) which contains the *lovA* gene disrupted by the phleomycin resistance gene. This plasmid was then digested with *KpnI* and *ApaI* and the resulting fragment was used to transform *A. terreus* ATCC20542 to Zeocin resistance. Transformants were screened for lovastatin production as described below (Method A). In one of the transformants, WMH1733, lovastatin production was abolished and two new compounds accumulated. Genomic DNA was prepared from this strain and from *A. terreus* ATCC20542, digested with *EagI*, run out on an agarose gel, and capillary blotted onto a nylon membrane. The membrane was hybridized with the 6 kb *ApaI* - *KpnI* fragment from pWHM1419 labelled using the Genius II kit using the conditions described previously. The wild-type strain had hybridizing bands at 2.0 kb, 1.9 kb and 1.1 kb. Mutant strain WMH1733 had hybridizing bands at 2.5 kb, 2.0 kb, 1.1 kb and 0.7 kb confirming the homologous integration of a single copy of the fragment from pWHM1419 at the *lovA* locus.

lovC

To disrupt the dehydrogenase-like gene, *lovC*, a 2 kb *Eco*RI - *Bgl*II fragment from pTPKS100 was ligated with a 1.7 kb *Eco*RI - *Sac*I fragment from pWHM1274 and *Bgl*II - *Sac*I digested litmus 28 (New England Biolabs) to produce pDH1 (pWHM1420). Another plasmid pDH2 (pWHM1421) was constructed from a 2.2 kb *Acc*65I - *Sac*I fragment from pWHM1274, a 2.1 kb *Hin*dIII - *Sac*I fragment from pPLOA containing the phleomycin resistance gene and *Hin*dIII - *Acc*65I digested litmus 28. The disruption vector pDH-dis (pWHM1422) was constructed by ligating together a 2.5 kb *Bgl*II - *Hpa*I fragment from pWHM1420, a 4.3 kb *Eco*RV - *Kpn*I fragment from pWHM1421 and *Bgl*II - *Kpn*I digested litmus 28. This plasmid was digested with *Bgl*II and *Kpn*I and the resulting 6.8 kb fragment was used to transform *A. terreus* ATCC20542 to Zeocin resistance. Transformants were screened for lovastatin production as described below (Method A). In two of the transformants, WMH1734 and WMH1735, lovastatin production was abolished. Genomic DNA was prepared from these strains and from *A. terreus* ATCC20542, digested with *Eag*I, run out on an agarose gel, and capillary blotted onto a nylon membrane. The membrane was hybridized with the 6.8 kb *Bgl* II- *Kpn*I fragment from pWHM1422 labelled using the Genius II kit using the conditions described previously. The wild type strain had hybridizing bands at 5 kb, 1.5 kb and 1.3 kb.

Mutant strain WMH1734 had hybridizing bands at 4.9 kb, 1.3 kb, 1.0 kb and 0.7 kb confirming the homologous integration of a single copy of the fragment from pWHM1422 at the *lovC* locus. The other mutant strain, WMH1735, had a similar banding pattern but with additional hybridizing bands indicating that multiple integration events had occurred, one of which was at the *lovC* locus.

Construction and characterization of the *A. terreus* strain with extra copies of *lovE*.

A 10.4 kb *Not*I- *Eco*RI fragment containing the putative regulatory gene, *lovE* was subcloned from pWHM1263 to pSPORT1 to give pWHM1276. From this plasmid a 3.9 kb *Hin*dIII - *Bam*HI fragment was subcloned into pGEM7 to give pWHM1423. The regulatory gene was subcloned from this vector into pPLOA as an *Sst*I - *Xba*RI fragment to give pWHM1424 (Fig. 5). pWHM1424 contains nucleotides 30,055 - 33,000 from SEQ ID NO:18 and nucleotides 1 - 1,026 from SEQ ID NO:19.

Extra copies of the regulatory gene were introduced into *A. terreus* ATCC20542 by transformation to Zeocin resistance with pWHM1424. Transformants were fermented (method A) and screened for lovastatin production initially by TLC analysis. Most of the transformants appeared to be producing significantly more lovastatin than the wild-type strain. The yields of lovastatin from the two transformant strains, WMH1736 and WMH1737, which had the most elevated levels compared to the wild-type

was quantified by HPLC as described below. These were found to produce 7-fold and 5-fold more lovastatin than the *A. terreus* ATCC20542 strain.

Because of the way that the DNA integrates (ectopically), each transformant is or can be unique, genotypically and phenotypically. However, some will be overproducers; others may exhibit no difference, for unknown reasons.

Heterologous expression of the lovastatin biosynthesis genes.

To place the NPKS gene (*lovB*) under the control of the inducible *alcA* promoter, the 11.5 kb *KpnI* - *AvrII* fragment from pTPKS100 containing the NPKS open reading frame was ligated into pAL3 (Waring, et al., Gene 79:119, 1989) previously digested with *KpnI* and *XbaI*. The resulting plasmid was designated pAL3TPKS (WHM1425). The polymerase chain reaction was used to amplify the NPKS gene sequence between the NPKS promoter region just upstream of the translational start codon and a *AgeI* site internal to NPKS. The design of the forward primer introduced a *KpnI* site 31 bases from the translational start codon allowing the NPKS to be placed against the *alcA* promoter but also incorporating upstream elements from the *A. terreus* system. Amplification was performed using Vent DNA polymerase with pTPKS100 as template and 1 μmol of each primer in a final volume of 100 μl using the manufacturer's buffer recommendations. After an initial

denaturation cycle of 10 minutes at 95°C amplification was achieved with 30 cycles of 95°C for 1 minute; 55°C for 1 minute and 72°C for 1.5 minutes. The final cycle was followed by 10 minutes at 72°C to ensure complete polymerization. The amplified product (1.7 kb) was digested with *KpnI* and *AgeI* and ligated into pWHM1425 that had been digested with the same enzymes and gel isolated. The resulting plasmid was designated pAL3TPKSNT (pWHM1426). The region introduced by PCR was sequenced on a ABI automated DNA sequencer to ensure sequence fidelity. This plasmid was then used to transform *A. nidulans* strain A722 (Fungal Genetics Stock Centre) to uridine prototrophy.

Transformants were grown by inoculating 0.5 ml of spore suspension (10^8 c.f.u./ml) in 50 ml YEPD in a 250 ml unbaffled flask. This was then grown for 20 hours at 250 rpm and 37°C (New Brunswick Scientific Series 25 Incubator Shaker). The mycelia were then harvested by filtration through Miracloth (Calbiochem), rinsed with sterile, distilled water, and inoculated into fresh 250 ml unbaffled flasks containing 50 ml AMM + lactose + 10 mM cyclopentanone and grown for a further 20 hours under the same conditions. The mycelia were harvested by filtration using Miracloth (Calbiochem), squeezed as dry as possible and frozen in liquid nitrogen. Protein extracts for SDS-PAGE and western analysis were prepared as described in Kennedy and Turner, Molec. Gen. Genet. (1996), 253:189-197, 1996.

One transformant, WMH1738, was shown to have a large protein (>200 kDa) visible on a SDS-PAGE gel that cross reacted with the affinity purified NPKS antibodies (Panlabs). This strain WMH1738 was transformed to hygromycin B resistance with pWHM1263. Transformant colonies were screened for lovastatin resistance and for the production of new metabolites as described below and two strains WMH1739 and WMH1740 were chosen for further analysis. Both of these strains were found to be significantly resistant (up to 100 μg/ml on solid media) to lovastatin compared with the host strain. This was analyzed by streaking 10 μl of a spore suspension on solid AMM plates containing lovastatin at 0, 0.1, 0.5, 1, 5, 10, 50 and 100 μg/ml and incubating at 37°C. Strains WMH1739 and WMH1740 were compared to strains WMH1741 and WMH1742 which were derivatives of WMH1738 transformed to hygromycin resistance with AN26. Strains WMH1739 and -1740 exhibited no inhibition of growth at any of these lovastatin concentrations whereas strains WMH1741 and -1742 showed slight inhibition of grown at 5 μg/ml and almost complete growth inhibition at 50 μg/ml. The two lovastatin resistant strains were fermented in lovastatin-producing conditions using fermentation method B and extracts were analyzed for lovastatin related metabolites as described below. Both strains were found to produce new metabolites. One compound that was common to both comigrated with monacolin J on TLC and HPLC analysis by the methods described below. Semi-

preparative HPLC was used to partially purify some of this compound, which was then analyzed by HPLC - MS. It had the same mass and fragmentation pattern as authentic monacolin J. The other compound, found in only one of the strains, comigrated with monacolin L on TLC and HPLC.

METHODS

Solid medium for growth of *A. terreus*

For the generation of spore suspensions *A. terreus* strains were grown on CM agar at 30°C for 4 to 5 days.

CM Agar (for CM liquid medium the agar was omitted):
50 ml Clutterbuck's salts (Vinci, et al., U.S. Patent No. 5,744,350)
2 ml Vogel's trace elements (Vinci, et al., U.S. Patent No. 5,744,350)
0.5% Difco Bacto tryptone
0.5% Difco Bacto yeast extract
1% glucose
2% Difco Bacto agar
in 1 liter of distilled water

Clutterbuck's salts:

12% $NaNO_3$
1.02% KCl
1.04% $MgSO_4.7H_2O$
3.04% KH_2PO_4

Vogel's trace elements:

0.004% $ZnCl_2$
0.02% $FeCl_3$
0.001% $CuCl_2$
0.001% $MnCl_2 \cdot 4H_2O$
0.001% $Na_2B_4O_7 \cdot 10H_2O$
0.001% $(NH_4)_6Mo_7O_{24} \cdot 7H_2O$

For long term storage *A. terreus* spores were suspended in SSS (10% glycerol, 5% lactose) and stored at -70°C.

For the generation of spore stocks *A. nidulans* was grown on the following solid growth medium (ACM) for 3 to 4 days at 37°C.

ACM:

2% Difco Bacto malt extract
0.1% Difco Bacto peptone
2% glucose
2% agar (Difco, Detroit, MI)

For strains which required para-aminobenzoic acid (PABA) for growth, PABA was added to a final concentration of 1 μg/ml. For strains which required uracil and uridine these were added at 20 mM and 10 mM, respectively. Spores were suspended in Tween 80 - saline solution (0.025% Tween 80, 0.8% NaCl) and stored at 4°C.

AMM:

0.6% (w/v) $NaNO_3$
0.052% (w/v) KCl
0.152% (w/v) KH_2PO_4
0.052% (w/v) $MgSO_4 \cdot 7H_2O$
1% (w/v) glucose
0.1% (v/v) AMM trace elements solution
pH to 6.5 and make up to 1 liter with distilled water.

For preparation of plates 2% (w/v) Difco Bacto agar was added. If required the glucose can be omitted and an alternative carbon source (e.g., lactose added at the same concentration). For the preparation of transformation plates KCl was added at 4.47% (w/v) (0.6 M).

AMM trace elements solution:

0.1% (w/v) $FeSO_4 \cdot 7H_2O$
0.88% (w/v) $ZnSO_4 \cdot 7H_2O$
0.04% (w/v) $CuSO_4 \cdot 5H_2O$
0.015% (w/v) $MnSO_4 \cdot 4H_2O$
0.01% (w/v) $Na_2B_4O_7 \cdot 10H_2O$

0.005% $(NH_4)_6Mo_7O_{24} \cdot 7H_2O$
distilled water to 1 liter

Large scale genomic DNA preparation from *A. terreus* for genomic library construction.

A 2.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 500 ml of liquid CM medium and grown for 20 hours at 30°C and 200 rpm. The mycelium was harvested by filtration through Miracloth (Calbiochem) and rinsed extensively with water then TSE [150 mM NaCl, 100 mM Na_2EDTA, 50 mM Tris-HCl pH 8.0]. The mycelium was squeezed dry, broken into small pellets and frozen in liquid nitrogen then ground to a fine powder in a pre-chilled pestle and mortar followed by transferral to a 500 ml flask. Fifty ml of extraction buffer [150 mM NaCl, 100 mM Na_2EDTA, 50 mM Tris-HCl pH 8.0, 2% (w/v) SDS] and 10 ml of toluene was added to the flask which was shaken at 60 rpm for 72 hours. This mixture was centrifuged at 1000 x g for 15 minutes and the supernatant was removed and extracted with an equal volume of chloroform:isoamyl alcohol (24:1 vol/vol). This mixture was centrifuged at 10,000 x g for 30 minutes at 15°C. The aqueous layer was carefully removed and 1.1 volumes of ethanol was layered on top. The DNA was spooled out from the resulting suspension and resuspended in 5 ml TE [10 mM Tris-HCl pH 8.0, 1 mM EDTA] + 50 μg/ml RNase and 100 μg/ml proteinase K then incubated at 37°C for 2 hours. The mixture was extracted again with chloroform:isoamyl alcohol (24:1) and the DNA was spooled out as before. Following resuspension in 1 ml of TE the

DNA was extracted once with phenol:chloroform:isoamyl alcohol (25:24:1, vol/vol), once with chloroform:isoamyl alcohol (24:1) and precipitated with 0.6 volumes isopropanol. The DNA clot was removed, dried briefly and resuspended in 0.5 ml TE.

Small scale genomic DNA preparation from *A. terreus* for Southern blot.

A 0.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 100 ml of liquid CM and grown for 20 hours at 30°C and 200 rpm. The mycelium was harvested by filtration through Miracloth (Calbiochem) and rinsed extensively with water then TSE [150 mM NaCl, 100 mM Na_2EDTA, 50 mM Tris-HCl pH 8.0]. The mycelium was squeezed dry, broken into small pellets and frozen in liquid nitrogen. The mycelium was ground to a fine powder in a pre-chilled pestle and mortar and transferred to a mortar pre-heated to 65°C. Three ml of lysis buffer [0.5 M NaCl, 10 mM Tris-HCl pH 7.5, 10 mM EDTA, 1% (w/v) SDS] at 65°C was added and 0.3 ml of 10% (w/v) cetyltrimethylammonium bromide in 0.7 M NaCl. After thorough mixing to form a slurry, 3 ml of phenol:chloroform:isoamyl alcohol (25:24:1) was added. This mixture was transferred to a Corex tube and incubated at 65°C for 15 minutes. Following centrifugation at 12,000 x g for 15 minutes at 4°C the aqueous phase was carefully removed and re-extracted once with phenol, once with phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). The DNA was precipitated from the extract by

addition of 0.1 volume of 3 M sodium acetate pH 5 and 0.6 volumes isopropanol then collected by centrifugation (10,000 x g, 10 minutes, 4°C). After washing with 70% ethanol the pellet was briefly dried and resuspended in TE + RNase (50 µg/ml).

Transformation of *A. terreus*.

A 0.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 100 ml of liquid CM and grown for 20 hours at 30°C and 200 rpm. The mycelium was harvested by centrifugation at 2000 x g for 15 minutes at 4°C and washed twice with an aqueous solution containing 0.27 M $CaCl_2$ and 0.6 M NaCl. To produce protoplasts the washed mycelia was resuspended in 20 ml of the same solution containing 5 mg/ml Novozym 234 (NovoNordisk) and incubated at 30°C for 1 - 3 hours with gentle agitation. Protoplasts were separated from undigested mycelia by filtration through Miracloth (Calbiochem). The protoplast suspension was diluted with an equal volume of STC1700 [1.2 M sorbitol, 10 mM Tris-HCl pH 7.5, 35 mM NaCl] and incubated on ice for 10 minutes. The protoplasts were collected by centrifugation (2000 x g, 10 minutes, 4°C), washed with STC1700 and resuspended in 1 ml STC1700. Plasmid DNA, purified using Qiagen columns, (2 - 5 µg in 10 µl) was added to 150 µl of protoplast suspension and incubated at room temperature for 25 minutes. PEG solution [60% (w/v) polyethylene glycol 4000, 50 mM $CaCl_2$, 10 mM Tris-HCl pH 7.5] was added to the DNA/protoplasts mixture in three steps: 250 µl,

250 µl, and 850 µl with mixing after each addition. The suspension was incubated at room temperature for 25 minutes then diluted to 10 ml with STC1700. Protoplasts were collected by centrifugation as above and diluted with 500 µl STC1700. 100 µl aliquots of this mixture were plated onto osmotically stabilized plates [CM medium containing 3% (w/v) Difco Bacto agar and 23.4% (w/v) mannitol, 15 ml of agar per plate]. After 4 hours growth at 30°C, 25 ml of OL agar [1% (w/v) Difco Bacto peptone, 1% (w/v) Difco Bacto agar, 200 µg/ml Zeocin] was overlayered onto each dish. The plates were incubated for 3 - 4 days at 30°C before transformant colonies were picked. These were streaked to single colonies twice on selective media (CM + 100 µg/ml Zeocin) before spore suspensions were prepared.

Transformation of *A. nidulans*.

A 0.5 ml aliquot of spore suspension (10^8 c.f.u./ml) was used to inoculate 100 ml of YEPD [2% (w/v) Difco Bacto yeast extract, 2% (w/v) glucose, 0.1% Difco Bacto peptone] liquid medium including necessary supplements and grown for 20 hours at 37°C and 200 rpm. The mycelia was harvested by centrifugation (2000 x g, 10 minutes, 4°C) and washed twice with 0.6 M KCl. To generate protoplasts the mycelia was resuspended in 20 ml of 0.6 M KCl containing 5 mg/ml Novozym 234 and incubated at 30°C for 1 - 2 hours with gentle shaking. Protoplasts were separated from undigested mycelia by filtration through Miracloth (Calbiochem). The protoplasts were harvested

by centrifugation as described above and washed twice with 0.6 M KCl, then resuspended in 10 ml 0.6 M KCl + 50 mM $CaCl_2$. After counting in a haemocytometer the protoplasts were harvested by centrifugation as before and resuspended to a final concentration of 5 x 10^8 protoplasts/ml. To 50 µl of protoplast suspension, 5 µl of DNA (2 - 5 µg, purified using Qiagen columns) was added, then 12.5 µl of PEG solution [25% (w/v) PEG 6000, 50 mM $CaCl_2$, 10 mM Tris - HCl pH 7.5] and the mixture was incubated on ice for 20 minutes. A further 0.5 ml of PEG solution was added and the mixture was incubated on ice for a further 5 minutes. A 1 ml aliquot of 0.6 M KCl + 50 mM $CaCl_2$ was added and the protoplasts were plated out in 50 µl, 200 µl, and 400 µl aliquots. For transformation to uridine prototrophy, protoplasts were plated out onto AMM + 0.6 M KCl plates without adding uridine or uracil supplements. Plates were incubated at 37°C for 3 - 4 days when transformants were picked. For transformation to hygromycin B resistance protoplasts were plated out onto AMM + 0.6 M KCl plates (15 ml) and incubated for 4 hours at 30°C. 30 ml of 1% peptone, 1% agar, 1 mg/ml hygromycin B was then used to overlay the plates, which were incubated for 3 - 4 days when transformants were picked. Transformants from both methods were streaked out to single colonies on selective media (i.e., lacking uridine/uracil supplements or containing 1 µg/ml hygromycin B) twice before spore suspensions were made.

Analysis of strains for lovastatin production.

Two fermentation methods were used for the analysis of lovastatin production. In Method A, 0.5 ml of spore suspension (10^8 c.f.u./ml) was inoculated into 25 ml of SEED medium in 250 ml unbaffled flasks and grown for 18 hours at 250 rpm and 30°C (New Brunswick Scientific Model 25 incubator/shaker). A 1 ml portion of the resulting seed culture was used to inoculate 25 ml of FM in a 250 ml unbaffled flask and grown for 6 days in the conditions described above. Fermentation Method B involved inoculating 50 ml of RPM in a 250 ml unbaffled flask with 0.5 ml of spore suspension (10^8 c.f.u./ml) and growing at 30°C and 250 rpm for 7 days in a New Brunswick Scientific Series 25 Incubator Shaker.

SEED medium:

0.5% (w/v) Sigma corn steep liquor
4% (w/v) tomato paste
1% (w/v) oat flour
1% (w/v) glucose
1% (v/v) Vogel's trace elements
distilled water to 1 l

FM:

4.5% (w/v) glucose
2.4% (w/v) Sigma peptonized milk
0.25% (w/v) Difco Bacto yeast extract
0.25% (w/v) polyethylene glycol 2000
distilled water up to 1 l

RPM:

4% (w/v) lactose
0.3% (w/v) rapeseed meal
0.2% (w/v) KNO_3
0.3% (w/v) KH_2PO_4
0.05% (w/v) $MgSO_4 \cdot 7H_2O$
0.05% (w/v) NaCl
0.05% (v/v) Sigma antifoam B
0.05% (v/v)trace elements solution
pH to 6.5 and made up to 1 l with distilled water.

Trace elements solution is:

0.16% (w/v) $MnSO_4$
0.34% (w/v) $ZnSO_4 \cdot 7H_2O$
0.2% (w/v) $CoCl_2 \cdot 6H_2O$
0.5% (w/v) $FeSO_4 \cdot 7H_2O$

made up to 1 liter with distilled water.

The cultures were extracted by adjusting the pH of the media to 3 with HCl, adding an equal volume of ethyl acetate, and shaking the mixture on a New Brunswick Scientific Series 25 incubator/shaker at 250 rpm for 2 hours. For analysis, 1 ml of the ethyl acetate layer was dried under a nitrogen stream and resuspended in 0.1 ml of methanol. For TLC analysis 10 μl of this extract was run on C-18 reverse phase TLC plates (RP-18 F_{254} - Merck) in a solvent system of methanol:0.1% phosphoric acid (9:1). TLC plates were developed by spraying with 10% phosphomolybdic acid in methanol and heating with a heat gun. Extracts were compared with authentic lovastatin, monacolin J, monacolin L, and dihydromonacolin L (acid and lactone forms). For HPLC analysis a Waters Nova-Pak C_{18} (3.9 x 150 mm) column was used with a solvent system of acetonitrile (B) and 0.1% phosphoric acid (A). The column was eluted with a preprogrammed gradient of 0 to 100% B into A over 25 minutes using gradient 7 (Waters Millenium Software) with a flow rate of 1.5 ml/min and metabolites were detected with a Waters 996 Photodiode Array Detector; lovastatin was detected at 238 nm. For purification of metabolites a Waters Prep Nova-Pak HR C_{18} (7.8 x 300 mm) column was used. The same solvent system as above was used with gradient of 0 to 100% B in A over

75 minutes at a flow rate of 4.5 ml/min. Fractions were collected manually, back extracted with ethyl acetate and dried. For HPLC-MS an Aquapore OD-300 7 micron (1.0 x 100 mm) column was used with a gradient of 0 to 100% acetonitrile into A (0.05% TFA) over 30 minutes at a flow rate of 0.02 ml/min.

CLAIMS

We claim:

1. A method of increasing the production of lovastatin in a lovastatin-producing organism, comprising the steps of transforming the organism with the D4B segment, wherein the segment is transcribed and translated, and wherein an increase in lovastatin production occurs.

2. The method of claim 1 wherein the D4B segment is the *A. terreus* D4B segment.

3. The method of claim 1, wherein the D4B segment is identical to nucleotides 579 - 33,000 of SEQ ID NO:18 and 1 - 5,349 of SEQ ID NO:19.

4. The method of claim 1, wherein the lovastatin-producing organism is selected from the group consisting of *A. terreus* ATCC 20542 and ATCC 20541.

5. The method of claim 1, wherein the organism is selected from the group consisting of fungi and yeast.

6. The method of claim 1 wherein the increase is at least 2-fold.

7. The method of claim 1 wherein the nucleic acid sequence is identical to a sequence isolated from ATCC 98876.

8. The method of claim 1 additionally comprising transforming the organism with the entire *A. terreus* lovastatin gene cluster.

9. The method of claim 8 wherein the gene cluster comprises SEQ ID NOs:18 and 19.

10. The method of claim 8 wherein the nucleic acid sequence of the gene cluster is identical to sequences isolated from ATCC 98876 and 98877.

11. A method of increasing the production of monacolin J in a lovastatin-producing organism, comprising the steps of transforming the organism with the D4B segment, wherein the segment is translated, and wherein an increase monacolin J production occurs.

12. A method of increasing the production of lovastatin in a lovastatin-producing organism, comprising the step of transforming the organism with the LovE gene, wherein the nucleic acid sequence is translated, and wherein an increase in lovastatin production occurs.

13. The method of claim 12 wherein the increase is at least 2.0-fold.

14. The method of claim 13 wherein the increase is at least 5-fold.

15. The method of claim 12 wherein the nucleotide sequence of the LovE gene comprises SEQ ID NO:27.

16. A method of increasing the production of lovastatin in a lovastatin-producing organism comprising the steps of transforming the organism with a nucleic acid sequence comprising a truncated version of the *A. terreus* D4B segment, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

17. A method of increasing the production of lovastatin in a lovastatin-producing organism comprising the steps of transforming the organism with a nucleic acid sequence comprising a truncated version of the *A. terreus* lovastatin-producing gene cluster, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

18. A method of increasing or conferring the production of monacolin J in a non-lovastatin-producing organism comprising the steps of transforming the organism with a nucleic acid sequence comprising the D4B segment, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in monacolin J production occurs.

19. The method of claim 18 wherein the D4B segment is the A. *terreus* D4B segment.

20. The method of claim 18 wherein the D4B segment comprises nucleotides 579 - 33,000 of SEQ ID NO:18 and 1-5,349 of SEQ ID NO:19.

21. The method of claim 18 additionally comprising the step of converting the monacolin J into lovastatin.

22. The method of claim 18 additionally comprising the step of transforming the organism with a nucleic acid sequence comprising the LovF gene, wherein the nucleic acid sequence is transcribed and translated and wherein an increase in lovastatin production occurs.

23. An isolated nucleic acid sequence selected from the group consisting of SEQ ID NOs:20 - 36.

24. A lovastatin-producing organism, wherein the organism has been genetically modified to have increased lovastatin production, wherein the increase is at least 2-fold.

25. The organism of claim 24, wherein the organism is a yeast or a fungi.

26. A non-lovastatin producing organism, wherein the organism has been genetically modified to produce monacolin J.

27. The organism of claim 26, wherein the organism is a yeast or a fungi.

28. A non-lovastatin producing organism, wherein the organism has been genetically modified to produce lovastatin.

29. The organism of claim 28 wherein the organism is a yeast or a fungi.

Lovastatin production genes

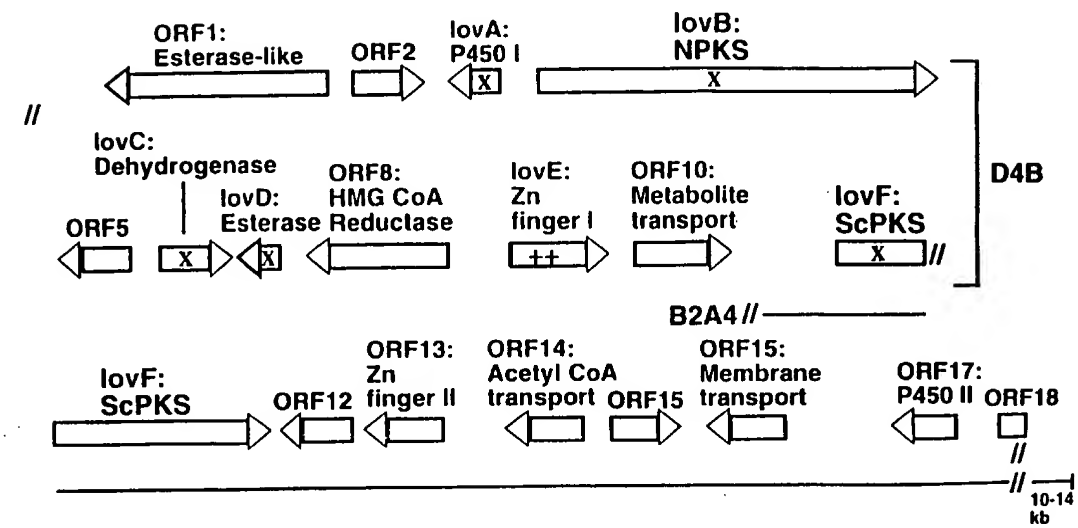


Fig. 1

WO 00/37629

PCT/US99/29583

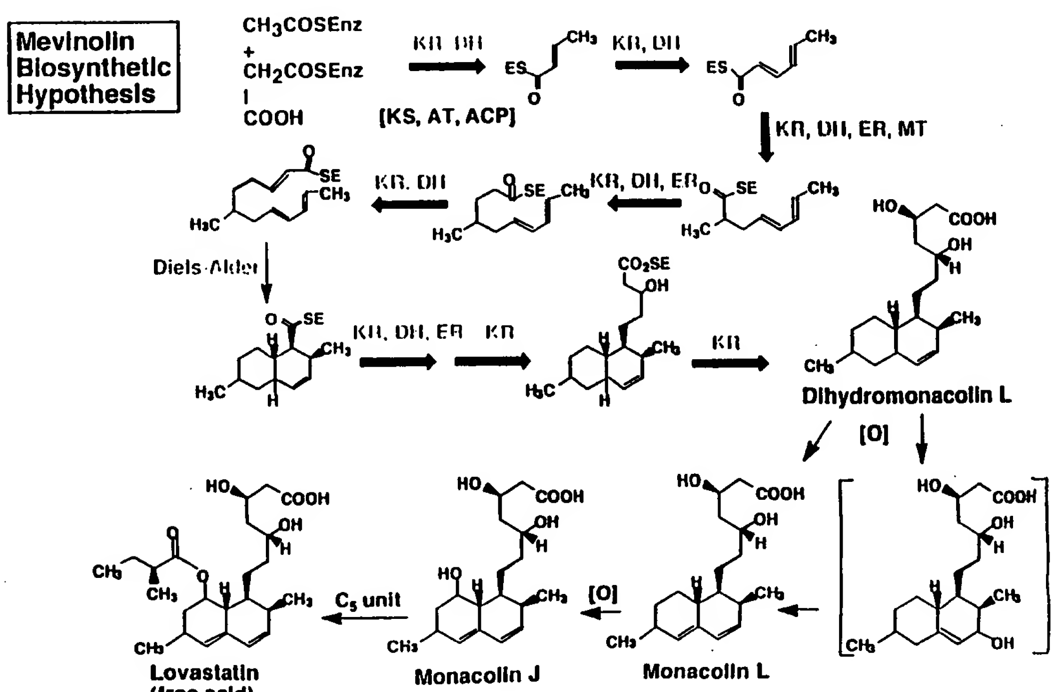


FIG. 2

The "Statins"

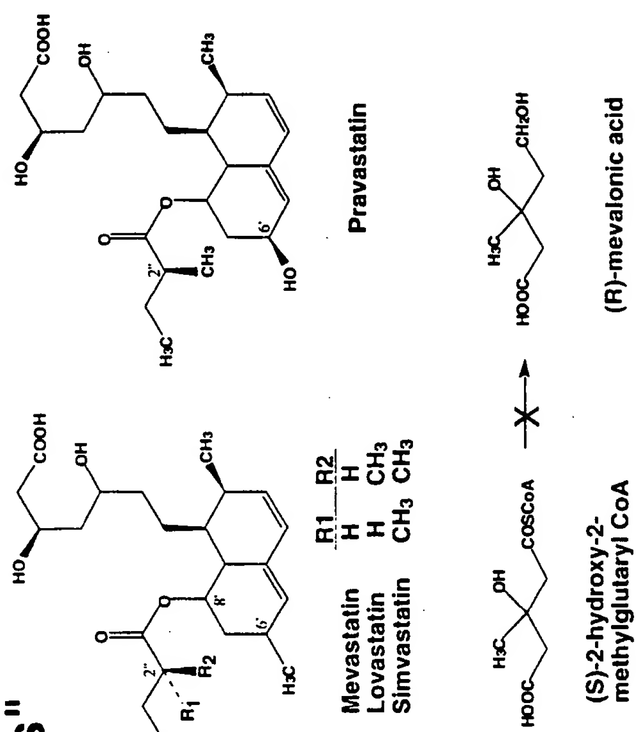


FIG. 3

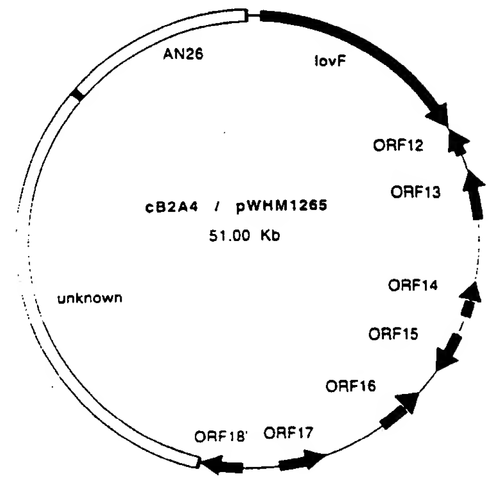
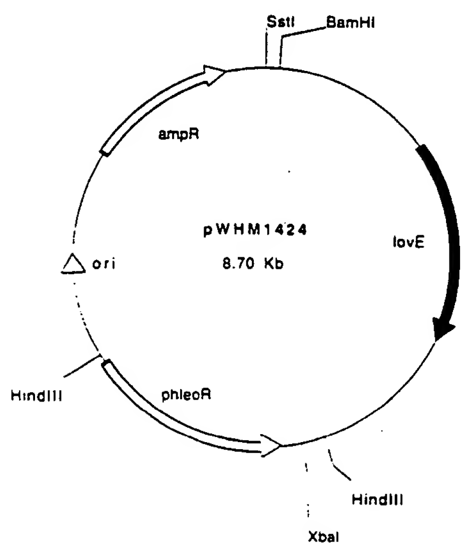


FIG. 4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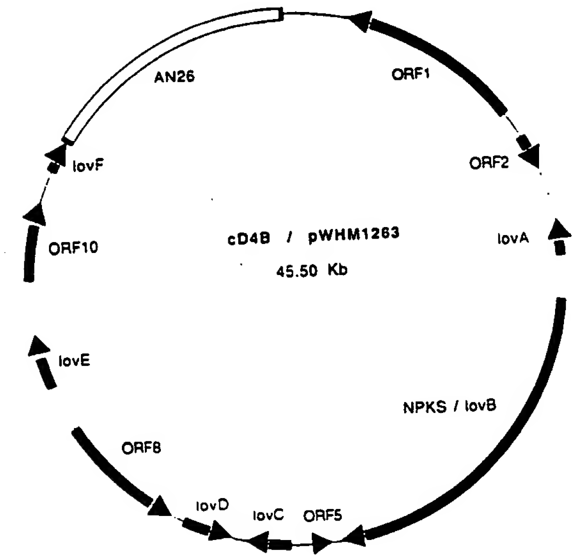
XbaI

FIG. 5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ORF2
lovF
cD4B / pWHM1263
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lovA
ORF10
lovE
NPKS / lovB
ORF8
lovD
lovC
ORF5

FIG. 6

SEQUENCE LISTING

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      Hutchinson, Charles R.
      Kennedy, Jonathan n.m.i
      Park, Cheonseck n.m.i

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         35                  40                  45

Ala Lys Arg Asp Asp Ala Leu Lys Val Pro Leu Arg Ile Leu Pro Leu
     50                  55                  60

Gly Ala Ser Ile Thr Trp Gly Tyr Leu Ser Ser Thr Gly Asn Gly Tyr
 65                  70                  75                  80

Arg Lys Pro Leu Arg Asp Lys Leu Arg Phe Glu Gly Trp Glu Val Asp
                 85                  90                  95

Met Val Gly Lys Ala His Ser Gly Asp Val Ile Thr Gln Val Gln Thr
            100                 105                 110

Ala Ala Ala Asn Ser Leu Ala Tyr Lys Pro Asn Val Val Leu Ile Asn
        115                 120                 125

Ala Gly Thr Asn Asp Cys Asp Tyr Asn Val Asp Pro Ala Asn Ala Gly
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Glu Arg Met Arg Ser Leu Ile Glu Thr Leu Ile Gly Ala Pro Asp Met
145                 150                 155                 160

Ala Asn Thr Leu Ile Val Leu Ser Thr Leu Ile Pro Ser Gly Ser Thr
                165                 170                 175

Thr Leu Glu Ala Asn Arg Pro Ser Val Asn Ala Gln Phe Arg Glu Leu
            180                 185                 190

Val Leu Asp Met Arg Glu Ala Gln Asn Val Ser Ile Val Leu Ala Asp
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Met Ala Asp Ile Trp Tyr Asn Ala Ile Tyr Asn Ala Ala Val Ala Glu
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Leu Ile Val Lys Pro Ala Asp Leu Asp Ile Ser Ser Thr Gly Thr Cys
            260                 265                 270

Asp Lys Glu Tyr Gly Ser Gly Val Tyr Ala Gly Gly Phe Thr Gln Gln
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Gly Ser Gly Glu Asp Asp Gly Ile Tyr Arg His Asp Ser Glu Tyr Ser
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Gly Ala Leu Phe Thr Val Arg Ala Gly Lys Gly Ala Ala Asp Pro Tyr
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Phe Thr Lys Gly Gly Thr Phe Ser Thr His Asn Asn Cys Asn Pro Gly
    370                 375                 380

Gly Val His Phe Ile Asp Ile Asn Gly Asp Gly Leu Asp Asp Tyr Ile
385                 390                 395                 400

Cys Ile Ala Leu Asp Gly Thr Thr Tyr Ala Ser Ile Asn Asn Gly Asp
                405                 410                 415

Gly Asp Ala Lys Ser Asn Lys Pro Pro Ser Phe Thr Asp Ile Gly Leu
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465                 470                 475                 480

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Phe Ala Gly Ile Tyr Gly Thr Ser Asp Leu Leu Ile Gly Gly Leu Val
        115                 120                 125

Asp Ile Val Arg His Pro His Leu Leu Glu Pro Leu Arg Asp Glu Ile
    130                 135                 140

Arg Thr Val Ile Gly Gln Gly Gly Trp Thr Pro Ala Ser Leu Tyr Lys
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Leu Lys Leu Leu Asp Ser Cys Leu Lys Glu Ser Gln Arg Val Lys Pro
                165                 170                 175

Val Glu Cys Ala Thr Met Arg Ser Tyr Ala Leu Gln Asp Val Thr Phe
            180                 185                 190

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225                 230                 235                 240

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            260                 265                 270

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Ile Pro Ile Val Gly Ala Ile Gly Phe Ser Glu Gly Ala Leu Val Thr
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145                 150                 155                 160

Ser Gln Tyr Met Arg Asn Glu Val Met Lys Asn His Asp Asp Asp Asn
                165                 170                 175

Asp Ser Lys Asp Thr Glu Trp Gln Glu Glu Leu Val Ile Arg Ile Pro
            180                 185                 190

Thr Leu His Leu Gln Gly Arg Asp Asp Phe Ala Leu Ala Gly Ser Lys
        195                 200                 205

Met Leu Val Ala Arg His Phe Ser Pro Arg Glu Ala Gln Val Leu Glu
    210                 215                 220

Phe Ala Gly Gln His Gln Phe Pro Asn Arg Pro Arg Asp Val Leu Glu
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     50                  55                  60

Phe Leu Gly Thr Asp Tyr Ala Gly Thr Val Val Ala Val Gly Ser Asp
 65                  70                  75                  80

Val Thr His Ile Gln Val Gly Asp Arg Val Tyr Gly Ala Gln Asn Glu
                 85                  90                  95
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            100                 105                 110

Thr Arg Gly Arg Val Trp Ala Lys Ile Pro Lys Gly Leu Ser Phe Glu
        115                 120                 125

Gln Ala Ala Ala Leu Pro Ala Gly Ile Ser Thr Ala Gly Leu Ala Met
    130                 135                 140

Lys Leu Leu Gly Leu Pro Leu Pro Ser Pro Ser Ala Asp Gln Pro Pro
145                 150                 155                 160

Thr His Ser Lys Pro Val Tyr Val Leu Val Tyr Gly Gly Ser Thr Ala
                165                 170                 175

Thr Ala Thr Val Thr Met Gln Met Leu Arg Leu Ser Gly Tyr Ile Pro
            180                 185                 190

Ile Ala Thr Cys Ser Pro His Asn Phe Asp Leu Ala Lys Ser Arg Gly
        195                 200                 205

Ala Glu Glu Val Phe Asp Tyr Arg Ala Pro Asn Leu Ala Gln Thr Ile
    210                 215                 220

Arg Thr Tyr Thr Lys Asn Asn Leu Arg Tyr Ala Leu Asp Cys Ile Thr
225                 230                 235                 240

Asn Val Glu Ser Thr Thr Phe Cys Phe Ala Ala Ile Gly Arg Ala Gly
                245                 250                 255

Gly His Tyr Val Ser Leu Asn Pro Phe Pro Glu His Ala Ala Thr Arg
            260                 265                 270

Lys Met Val Thr Thr Asp Trp Thr Leu Gly Pro Thr Ile Phe Gly Glu
        275                 280                 285

Gly Ser Thr Trp Pro Ala Pro Tyr Gly Arg Pro Gly Ser Glu Glu Glu
    290                 295                 300

Arg Gln Phe Gly Glu Asp Leu Trp Arg Ile Ala Gly Gln Leu Val Glu
305                 310                 315                 320

Asp Gly Arg Leu Val His His Pro Leu Arg Val Val Gln Gly Gly Phe
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Ala Val Ile Met Ala Arg Asp Cys Ser Gly Asn Leu Asn Tyr Thr Arg
        35                  40                  45
```



```
Cys Phe Gly Ala Arg Thr Val Arg Arg Asp Glu Cys Asn Gly Leu Pro
    50                  55                  60

Pro Leu Gln Val Asp Thr Pro Cys Arg Leu Ala Ser Ala Thr Lys Leu
65                  70                  75                  80

Leu Thr Thr Ile Met Ala Leu Gln Cys Met Glu Arg Gly Leu Val Asp
                85                  90                  95

Leu Asp Glu Thr Val Asp Arg Leu Leu Pro Asp Leu Ser Ala Met Pro
            100                 105                 110

Val Leu Glu Gly Phe Asp Asp Ala Gly Asn Ala Arg Leu Arg Glu Arg
        115                 120                 125

Arg Gly Lys Ile Thr Leu Arg His Leu Leu Thr His Thr Ser Gly Leu
    130                 135                 140

Ser Tyr Val Phe Leu His Pro Leu Leu Arg Glu Tyr Met Ala Gln Gly
145                 150                 155                 160

His Leu Gln Ser Ala Glu Lys Phe Gly Ile Glx Ser Arg Leu Ala Pro
                165                 170                 175

Pro Ala Val Asn Asp Pro Gly Ala Glu Trp Ile Tyr Gly Ala Asn Leu
            180                 165                 190

Asp Trp Ala Gly Lys Leu Val Glu Arg Ala Thr Gly Leu Asp Leu Glu
        195                 200                 205

Gln Tyr Leu Gln Glu Asn Ile Cys Ala Pro Leu Gly Ile Thr Asp Met
    210                 215                 220

Thr Phe Lys Leu Gln Gln Arg Pro Asp Met Leu Ala Arg Arg Ala Asp
225                 230                 235                 240

Gln Thr His Arg Asn Ser Ala Asp Gly Arg Leu Arg Tyr Asp Asp Ser
                245                 250                 255

Val Tyr Phe Arg Ala Asp Gly Glu Glu Cys Phe Gly Gly Gln Gly Val
            260                 265                 270

Phe Ser Gly Pro Gly Ser Tyr Met Lys Val Leu His Ser Leu Leu Lys
        275                 280                 285

Arg Asp Gly Leu Leu Leu Gln Pro Gln Thr Val Asp Leu Met Phe Gln
    290                 295                 300

Pro Ala Leu Glu Pro Arg Leu Glu Glu Gln Met Asn Gln His Met Asp
305                 310                 315                 320

Ala Ser Pro His Ile Asn Tyr Gly Gly Pro Met Pro Met Val Leu Arg
                325                 330                 335

Arg Ser Phe Gly Leu Gly Gly Ile Ile Ala Leu Glu Asp Leu Asp Gly
            340                 345                 350

Glu Asn Trp Arg Arg Lys Gly Ser Leu Thr Phe Gly Gly Gly Pro Asn
        355                 360                 365

Ile Val Trp Gln Ile Asp Pro Lys Ala Gly Leu Cys Thr Leu Ala Phe
    370                 375                 380

Phe Gln Leu Glu Pro Trp Asn Asp Pro Val Cys Arg Asp Leu Thr Arg
385                 390                 395                 400
```

```
Thr Phe Glu His Ala Ile Tyr Ala Gln Tyr Gln Gln Gly
                405                 410

<210> 7
<211> 1068
<212> PRT
<213> Aspergillus terreus

<400> 7
Met Asp Pro Val Val Arg Lys Pro Asp Pro Gly Gly Val Gln His Arg
1               5                   10                  15

Val Thr Lys Ala Leu Arg Ala Ile Val Gly His Ala Cys Arg His Pro
            20                  25                  30

Ile His Thr Leu Leu Val Thr Ala Leu Thr Ala Ala Thr Thr His Leu
        35                  40                  45

His Val Leu Glu Gly Thr Tyr Gln Ala Thr His Arg Glu Ala Ser Ala
    50                  55                  60

Trp Lys Trp Gln Ile Asp Asp Arg Pro Lys Val Pro Glu Asp Gly Gln
65                  70                  75                  80

Ser Asp Phe His Trp Ala Leu Val Thr Leu Asp Leu Pro Gly Ala Ser
                85                  90                  95

Val Asp Ala Ser Ile Pro Phe Leu Ser Asn Thr Leu Ser Gly Phe Leu
            100                 105                 110

Gly Ala Glu Gln Thr Thr Pro Thr Pro Asp Ser Ser Pro Ser Pro Asp
        115                 120                 125

His Ser Ala Leu Thr Phe Arg Val Pro Tyr Ser Gln Leu Asp Gly Phe
    130                 135                 140

Leu Gln Ala Val Glu Ile Ile Pro Ser Glu Lys Glu Asp Asp Ser Trp
145                 150                 155                 160

Arg Leu Arg Ser Pro Arg Glu Glu Gly Ser Pro Arg Ser Leu Gly His
                165                 170                 175

Trp Leu Gly Ser Ser Trp Leu Ser Phe Leu His Arg Val His His Ala
            180                 185                 190

Glu Thr Val Asp Leu Val Ile Ile Gly Leu Ser Tyr Leu Ala Met Asn
        195                 200                 205

Met Thr Val Val Ser Leu Phe Arg Val Met Arg His Leu Gly Ser Arg
    210                 215                 220

Phe Trp Leu Ala Ala Ser Val Leu Leu Ser Gly Ala Phe Ala Phe Val
225                 230                 235                 240

Leu Gly Leu Gly Ile Thr Thr Thr Cys Asp Val Pro Val Asp Met Leu
                245                 250                 255

Leu Leu Phe Glu Gly Ile Pro Tyr Leu Val Leu Thr Val Gly Phe Glu
            260                 265                 270

Lys Pro Ile Gln Leu Thr Arg Ala Val Leu Cys Val Ser Glu Glu Leu
        275                 280                 285

Trp Gly Gly Gly Gln Arg Gln Val Pro Asn Gly Ala Ser Ser Asp Asp
    290                 295                 300
```



```
Ser Arg Gln Asn Gln Leu Ile Pro Asn Ile Ile Gln Leu Ala Val Asp
305                 310                 315                 320

Arg Glu Gly Trp Tyr Ile Val Arg Ser Tyr Leu Leu Glu Ile Gly Ala
                325                 330                 335

Leu Ala Leu Gly Ala Val Leu Arg Pro Lys Asp Ser Leu Gly His Phe
            340                 345                 350

Cys Phe Leu Ala Ala Trp Thr Leu Leu Ile Asp Ala Val Leu Leu Phe
        355                 360                 365

Thr Phe Tyr Ala Thr Ile Leu Cys Val Lys Leu Glu Ile Thr Arg Ile
    370                 375                 380

Arg Ser Pro Gly Gly Leu Gly Gln Val Asn Ala Lys His Pro Ser Gly
385                 390                 395                 400

Ile Phe Gly His Lys Val Lys Ser Thr Asn Ile Thr Trp Trp Lys Leu
                405                 410                 415

Leu Thr Val Gly Gly Phe Val Leu Cys His Phe Leu Gln Leu Ser Pro
            420                 425                 430

Phe Phe Tyr Arg Val Met Gly Glu Tyr Met Ala Asn Gly Thr Leu Pro
        435                 440                 445

Pro Thr Ala Val Ser Pro Phe Lys Glu Ala Ala Asn Gly Leu Asn Glu
    450                 455                 460

Ile Tyr Leu Thr Ala Arg Val Glu Gly Phe Glu Thr Arg Val Thr Val
465                 470                 475                 480

Leu Pro Pro Leu Gln Tyr Val Leu Glu Ser Ala Gly Phe Asn Ile Ser
                485                 490                 495

Ala Thr Lys Arg Ser Thr Phe Asp Gly Val Leu Asp Gly Leu Glu Ser
            500                 505                 510

Pro Leu Gly Arg Leu Cys Leu Met Gly Ala Leu Val Val Ser Leu Val
        515                 520                 525

Leu Asn Asn His Leu Ile His Ala Ala Arg Trp His Ala Trp Pro Gln
    530                 535                 540

Ala Arg Glu Ser Ala Val Pro Asp Gly Ser Tyr Leu Ser Val Pro Cys
545                 550                 555                 560

Ser Ala Thr Ala Pro Glu Val Cys Thr Arg Pro Pro Glu Glu Thr Glu
                565                 570                 575

Ala Leu Leu Lys Ser Asn Gln Ala Glu Ser Leu Thr Asp Asp Glu Leu
            580                 585                 590

Val Glu Leu Cys Leu Arg Gly Lys Ile Ala Gly Tyr Ser Leu Glu Lys
        595                 600                 605

Thr Leu Glu Arg Ile Ala Ala Gly Ser Ser Arg Ser Val Thr Arg Leu
    610                 615                 620

Glu Ala Phe Thr Arg Ala Val Arg Ile Arg Arg Ala Ala Val Ser Lys
625                 630                 635                 640

Thr Pro Ser Thr Gln Asn Leu Cys Ser Gly Leu Ala Glu Ser Leu Leu
                645                 650                 655
```

```
Pro Tyr Arg Asp Tyr Asn Tyr Glu Leu Val His Gly Ala Cys Cys Glu
            660                 665                 670

Asn Val Val Gly Tyr Leu Pro Leu Pro Leu Gly Val Ala Gly Pro Met
        675                 680                 685

Val Ile Asp Gly Gln Ala Leu Phe Ile Pro Met Ala Thr Thr Glu Gly
    690                 695                 700

Val Leu Val Ala Ser Ala Ser Arg Gly Cys Lys Ala Ile Asn Ala Gly
705                 710                 715                 720

Gly Gly Ala Thr Thr Met Leu Lys Gly Asp Gly Met Thr Arg Gly Pro
                725                 730                 735

Cys Leu Arg Phe Pro Ser Ala Gln Arg Ala Ala Glu Ala Gln Arg Trp
            740                 745                 750

Val Glu Ser Pro Leu Gly His Glu Val Leu Ala Ala Ala Phe Asn Ala
        755                 760                 765

Thr Ser Arg Phe Ala Arg Leu Gln Thr Leu Thr Val Ala Gln Ala Gly
    770                 775                 780

Ile Tyr Leu Tyr Ile Arg Phe Arg Thr Thr Thr Gly Asp Ala Met Gly
785                 790                 795                 800

Met Asn Met Ile Ser Lys Gly Val Glu Lys Ala Leu Glu Ala Met Ala
                805                 810                 815

Ala Glu Gly Gly Phe Pro Asp Met His Thr Val Thr Leu Ser Gly Asn
            820                 825                 830

Phe Cys Ser Asp Lys Lys Ser Ala Ala Ile Asn Trp Ile Gly Gly Arg
        835                 840                 845

Gly Lys Ser Val Ile Ala Glu Ala Thr Ile Pro Ala Glu Thr Val Arg
    850                 855                 860

Gln Val Leu Lys Thr Asp Val Asp Ala Leu Val Glu Leu Asn Thr Ala
865                 870                 875                 880

Lys Asn Leu Val Gly Ser Ala Met Ala Gly Ser Leu Gly Gly Phe Asn
                885                 890                 895

Ala His Ala Ser Asn Leu Val Gln Ala Val Phe Leu Ala Thr Gly Gln
            900                 905                 910

Asp Pro Ala Gln Asn Val Glu Ser Ser Ser Cys Ile Thr Thr Met Lys
        915                 920                 925

Asn Ile Asp Gly Asn Leu His Ile Ala Val Ser Met Pro Ser Met Glu
    930                 935                 940

Val Gly Thr Ile Gly Gly Gly Thr Ile Leu Glu Ala Gln Gly Ala Met
945                 950                 955                 960

Leu Asp Leu Leu Gly Val Arg Gly Ala His Ser Thr Glu Pro Gly Ala
                965                 970                 975

Asn Ala Arg Arg Leu Ala Arg Ile Val Ala Ala Ala Val Leu Ala Gly
            980                 985                 990

Glu Leu Ser Thr Cys Ala Ala Leu Ala Ala Gly His Leu Val Asn Ala
        995                 1000                1005
```



```
His Met Gln His Asn Arg Thr Ser Lys Asp Ala Ile Ser Gly Thr Glu
    1010                1015                1020

Tyr Gly Ala Ile Arg Thr Pro Val Tyr Val Val Ile Leu Glu His Ala
1025                1030                1035                1040

Gly Asp Ile His Phe Val Gln Ile Glu Tyr Lys Asn Thr Tyr Leu Arg
                1045                1050                1055

Arg Lys Val Pro Thr Leu Ser Cys Asn Leu Gly Arg
            1060                1065
```

```
<210> 8
<211> 503
<212> PRT
<213> Aspergillus terreus

<400> 8
Met Ala Ala Asp Gln Gly Ile Phe Thr Asn Ser Val Thr Leu Ser Pro
1               5                   10                  15

Val Glu Gly Ser Arg Thr Gly Gly Thr Leu Pro Arg Arg Ala Phe Arg
            20                  25                  30

Arg Ser Cys Asp Arg Cys His Ala Gln Lys Ile Lys Cys Thr Gly Asn
        35                  40                  45

Lys Glu Val Thr Gly Arg Ala Pro Cys Gln Arg Cys Gln Gln Ala Gly
    50                  55                  60

Leu Arg Cys Val Tyr Ser Glu Arg Cys Pro Lys Arg Lys Leu Arg Gln
65                  70                  75                  80

Ser Arg Ala Ala Asp Leu Val Ser Ala Asp Pro Asp Pro Cys Leu His
                85                  90                  95

Met Ser Ser Pro Pro Val Pro Ser Gln Ser Leu Pro Leu Asp Val Ser
            100                 105                 110

Glu Ser His Ser Ser Asn Thr Ser Arg Gln Phe Leu Asp Pro Pro Asp
        115                 120                 125

Ser Tyr Asp Trp Ser Trp Thr Ser Ile Gly Thr Asp Glu Ala Ile Asp
    130                 135                 140

Thr Asp Cys Trp Gly Leu Ser Gln Cys Asp Gly Gly Phe Ser Cys Gln
145                 150                 155                 160

Leu Glu Pro Thr Leu Pro Asp Leu Pro Ser Pro Phe Glu Ser Thr Val
                165                 170                 175

Glu Lys Ala Pro Leu Pro Pro Val Ser Ser Asp Ile Ala Arg Ala Ala
            180                 185                 190

Ser Ala Gln Arg Glu Leu Phe Asp Asp Leu Ser Ala Val Ser Gln Glu
        195                 200                 205

Leu Glu Glu Ile Leu Leu Ala Val Thr Val Glu Trp Pro Lys Gln Glu
    210                 215                 220

Ile Trp Thr Arg Ala Ser Pro His Ser Pro Thr Ala Ser Arg Glu Arg
225                 230                 235                 240

Ile Ala Gln Arg Arg Gln Asn Val Trp Ala Asn Trp Leu Thr Asp Leu
                245                 250                 255
```

```
His Met Phe Ser Leu Asp Pro Ile Gly Met Phe Phe Asn Ala Ser Arg
            260                 265                 270

Arg Leu Leu Thr Val Leu Arg Gln Gln Ala Gln Ala Asp Cys His Gln
        275                 280                 285

Gly Thr Leu Asp Glu Cys Leu Arg Thr Lys Asn Leu Phe Thr Ala Val
    290                 295                 300

His Cys Tyr Ile Leu Asn Val Arg Ile Leu Thr Ala Ile Ser Glu Leu
305                 310                 315                 320

Leu Leu Ser Gln Ile Arg Arg Thr Gln Asn Ser His Met Ser Pro Leu
                325                 330                 335

Glu Gly Ser Arg Ser Gln Ser Pro Ser Arg Asp Asp Thr Ser Ser Ser
            340                 345                 350

Ser Gly His Ser Ser Val Asp Thr Ile Pro Phe Phe Ser Glu Asn Leu
        355                 360                 365

Pro Ile Gly Glu Leu Phe Ser Tyr Val Asp Pro Leu Thr His Ala Leu
    370                 375                 380

Phe Ser Ala Cys Thr Thr Leu His Val Gly Val Gln Leu Leu Arg Glu
385                 390                 395                 400

Asn Glu Ile Thr Leu Gly Val His Ser Ala Gln Gly Ile Ala Ala Ser
                405                 410                 415

Ile Ser Met Ser Gly Glu Pro Gly Glu Asp Ile Ala Arg Thr Gly Ala
            420                 425                 430

Thr Asn Ser Ala Arg Cys Glu Glu Gln Pro Thr Thr Pro Ala Ala Arg
        435                 440                 445

Val Leu Phe Met Phe Leu Ser Asp Glu Gly Ala Phe Gln Glu Ala Lys
    450                 455                 460

Ser Ala Gly Ser Arg Gly Arg Thr Ile Ala Ala Leu Arg Arg Cys Tyr
465                 470                 475                 480

Glu Asp Ile Phe Ser Leu Ala Arg Lys His Lys His Gly Met Leu Arg
                485                 490                 495

Asp Leu Asn Asn Ile Pro Pro
            500


<210> 9
<211> 542
<212> PRT
<213> Aspergillus terreus

<400> 9
Met Thr Ser His His Gly Glu Thr Glu Lys Pro Gln Ser Asn Thr Ala
 1               5                  10                  15

Gln Met Gln Ile Asn His Val Thr Gly Leu Arg Leu Gly Leu Val Val
             20                  25                  30

Val Ser Val Thr Leu Val Ala Phe Leu Met Leu Leu Asp Met Ser Ile
         35                  40                  45

Ile Val Thr Ala Ile Pro His Ile Thr Ala Gln Phe His Ser Leu Gly
     50                  55                  60
```



```
Asp Val Gly Trp Tyr Gly Ser Ala Tyr Leu Leu Ser Ser Cys Ala Leu
 65                  70                  75                  80

Gln Pro Leu Ala Gly Lys Leu Tyr Thr Leu Leu Thr Leu Lys Tyr Thr
                 85                  90                  95

Phe Leu Ala Phe Leu Gly Leu Phe Glu Ile Gly Ser Val Leu Cys Gly
            100                 105                 110

Thr Ala Arg Ser Ser Thr Met Leu Ile Val Gly Arg Ala Val Ala Gly
        115                 120                 125

Met Gly Gly Ser Gly Leu Thr Asn Gly Ala Ile Thr Ile Leu Ser Ala
    130                 135                 140

Ala Ala Pro Lys Gln Gln Gln Pro Leu Leu Ile Gly Ile Met Met Gly
145                 150                 155                 160

Leu Ser Gln Ile Ala Ile Val Cys Gly Pro Leu Leu Gly Gly Ala Phe
                165                 170                 175

Thr Gln His Ala Ser Trp Arg Trp Cys Phe Tyr Ile Asn Leu Pro Ile
            180                 185                 190

Gly Ala Phe Ala Thr Phe Leu Leu Leu Val Ile Gln Ile Pro Asn Arg
        195                 200                 205

Leu Pro Ser Thr Ser Asp Ser Thr Thr Asp Gly Thr Asn Pro Lys Arg
    210                 215                 220

Arg Gly Ala Arg Asp Val Leu Thr Gln Leu Asp Phe Leu Gly Phe Val
225                 230                 235                 240

Leu Phe Ala Gly Phe Ala Ile Met Ile Ser Leu Ala Leu Glu Trp Gly
                245                 250                 255

Gly Ser Asp Tyr Ala Trp Asn Ser Ser Val Ile Ile Gly Leu Phe Cys
            260                 265                 270

Ala Ala Gly Val Ser Leu Val Leu Phe Gly Cys Trp Glu Arg His Val
        275                 280                 285

Gly Gly Ala Val Ala Met Ile Pro Ile Ser Val Ala Ser Arg Arg Gln
    290                 295                 300

Val Trp Cys Ser Cys Phe Phe Leu Gly Phe Phe Ser Gly Ala Leu Leu
305                 310                 315                 320

Ile Phe Ser Tyr Tyr Leu Pro Ile Tyr Phe Gln Ala Val Lys Asn Val
                325                 330                 335

Ser Pro Thr Met Ser Gly Val Tyr Met Leu Pro Gly Ile Gly Gly Gln
            340                 345                 350

Ile Val Met Ala Ile Val Thr Gly Ala Ile Ile Gly Lys Thr Gly Tyr
        355                 360                 365

Tyr Val Pro Trp Ala Leu Ala Ser Gly Ile Leu Val Ser Ile Ser Ala
    370                 375                 380

Gly Leu Val Ser Thr Phe Gln Pro Glu Thr Ser Ile Ala Ala Trp Val
385                 390                 395                 400

Met Tyr Gln Phe Leu Gly Gly Val Gly Arg Gly Cys Gly Met Gln Thr
                405                 410                 415
```

```
Pro Val Val Ala Ile Gln Asn Ala Leu Pro Pro Gln Thr Ser Pro Ile
            420                 425                 430

Gly Ile Ser Leu Ala Met Phe Gly Gln Thr Phe Gly Gly Ser Leu Phe
        435                 440                 445

Leu Thr Leu Thr Glu Leu Val Phe Ser Asn Gly Leu Asp Ser Gly Leu
    450                 455                 460

Arg Gln Tyr Ala Pro Thr Leu Asn Ala Gln Glu Val Thr Ala Ala Gly
465                 470                 475                 480

Ala Thr Gly Phe Arg Gln Val Val Pro Ala Pro Leu Ile Ser Arg Val
                485                 490                 495

Leu Leu Ala Tyr Ser Lys Gly Val Asp His Ala Phe Tyr Val Ala Val
            500                 505                 510

Gly Ala Ser Gly Ala Thr Phe Ile Phe Ala Trp Gly Met Gly Arg Leu
        515                 520                 525

Ala Trp Arg Gly Trp Arg Met Gln Glu Lys Gly Arg Ser Glu
    530                 535                 540


<210> 10
<211> 2532
<212> PRT
<213> Aspergillus terreus

<400> 10
Met Thr Pro Leu Asp Ala Pro Gly Ala Pro Ala Pro Ile Ala Met Val
  1               5                  10                  15

Gly Met Gly Cys Arg Phe Gly Gly Gly Ala Thr Asp Pro Gln Lys Leu
            20                  25                  30

Trp Lys Leu Leu Glu Glu Gly Gly Ser Ala Trp Ser Lys Ile Pro Pro
        35                  40                  45

Ser Arg Phe Asn Val Gly Gly Val Tyr His Pro Asn Gly Gln Arg Val
    50                  55                  60

Gly Ser Met His Val Arg Gly Gly His Phe Leu Asp Glu Asp Pro Ala
65                  70                  75                  80

Leu Phe Asp Ala Ser Phe Phe Asn Met Ser Thr Glu Val Ala Ser Cys
                85                  90                  95

Met Asp Pro Gln Tyr Arg Leu Ile Leu Glu Val Val Tyr Glu Ala Leu
            100                 105                 110

Glu Ala Ala Gly Ile Pro Leu Glu Gln Val Ser Gly Ser Lys Thr Gly
        115                 120                 125

Val Phe Ala Gly Thr Met Tyr His Asp Tyr Gln Gly Ser Phe Gln Arg
    130                 135                 140

Gln Pro Glu Ala Leu Pro Arg Tyr Phe Ile Thr Gly Asn Ala Gly Thr
145                 150                 155                 160

Met Leu Ala Asn Arg Val Ser His Phe Tyr Asp Leu Arg Gly Pro Ser
                165                 170                 175

Val Ser Ile Asp Thr Ala Cys Ser Thr Thr Leu Thr Ala Leu His Leu
            180                 185                 190
```



```
Ala Ile Gln Ser Leu Arg Ala Gly Glu Ser Asp Met Ala Ile Val Ala
        195                 200                 205

Gly Ala Asn Leu Leu Leu Asn Pro Asp Val Phe Thr Thr Met Ser Asn
    210                 215                 220

Leu Gly Phe Leu Ser Ser Asp Gly Ile Ser Tyr Ser Phe Asp Ser Arg
225                 230                 235                 240

Ala Asp Gly Tyr Gly Arg Gly Glu Gly Val Ala Ala Ile Val Leu Lys
                245                 250                 255

Thr Leu Pro Asp Ala Val Arg Asp Gly Asp Pro Ile Arg Leu Ile Val
            260                 265                 270

Arg Glu Thr Ala Ile Asn Gln Asp Gly Arg Thr Pro Ala Ile Ser Thr
        275                 280                 285

Pro Ser Gly Glu Ala Gln Glu Cys Leu Ile Gln Asp Cys Tyr Gln Lys
    290                 295                 300

Ala Gln Leu Asp Pro Lys Gln Thr Ser Tyr Val Glu Ala His Gly Thr
305                 310                 315                 320

Gly Thr Arg Ala Gly Asp Pro Leu Glu Leu Ala Val Ile Ser Ala Ala
                325                 330                 335

Phe Pro Gly Gln Gln Ile Gln Val Gly Ser Val Lys Ala Asn Ile Gly
            340                 345                 350

His Thr Glu Ala Val Ser Gly Leu Ala Ser Leu Ile Lys Val Ala Leu
        355                 360                 365

Ala Val Glu Lys Gly Val Ile Pro Pro Asn Ala Arg Phe Leu Gln Pro
    370                 375                 380

Ser Lys Lys Leu Leu Lys Asp Thr His Ile Gln Ile Pro Leu Cys Ser
385                 390                 395                 400

Gln Ser Trp Ile Pro Thr Asp Gly Val Arg Arg Ala Ser Ile Asn Asn
                405                 410                 415

Phe Gly Phe Gly Gly Ala Asn Ala His Ala Ile Val Glu Gln Tyr Gly
            420                 425                 430

Pro Phe Ala Glu Thr Ser Ile Cys Pro Pro Asn Gly Tyr Ser Gly Asn
        435                 440                 445

Tyr Asp Gly Asn Leu Gly Thr Asp Gln Ala His Ile Tyr Val Leu Ser
    450                 455                 460

Ala Lys Asp Glu Asn Ser Cys Met Arg Met Val Ser Arg Leu Cys Asp
465                 470                 475                 480

Tyr Ala Thr His Ala Arg Pro Ala Asp Asp Leu Gln Leu Leu Ala Asn
                485                 490                 495

Ile Ala Tyr Thr Leu Gly Ser Arg Arg Ser Asn Phe Arg Trp Lys Ala
            500                 505                 510

Val Cys Thr Ala His Ser Leu Thr Gly Leu Ala Gln Asn Leu Ala Gly
        515                 520                 525

Glu Gly Met Arg Pro Ser Lys Ser Ala Asp Gln Val Arg Leu Gly Trp
    530                 535                 540
```

```
Val Phe Thr Gly Gln Gly Ala Gln Trp Phe Ala Met Gly Arg Glu Leu
545                 550                 555                 560

Ile Glu Met Tyr Pro Val Phe Lys Glu Ala Leu Leu Glu Cys Asp Gly
                565                 570                 575

Tyr Ile Lys Glu Met Gly Ser Thr Trp Ser Ile Ile Glu Glu Leu Ser
            580                 585                 590

Arg Pro Glu Thr Glu Ser Arg Val Asp Gln Ala Glu Phe Ser Leu Pro
        595                 600                 605

Leu Ser Thr Ala Leu Gln Ile Ala Leu Val Arg Leu Leu Trp Ser Trp
    610                 615                 620

Asn Ile Gln Pro Val Ala Val Thr Ser His Ser Ser Gly Glu Ala Ala
625                 630                 635                 640

Ala Ala Tyr Ala Ile Gly Ala Leu Thr Ala Arg Ser Ala Ile Gly Ile
                645                 650                 655

Ser Tyr Ile Arg Gly Ala Leu Thr Ala Arg Asp Arg Leu Ala Ser Val
            660                 665                 670

His Lys Gly Gly Met Leu Ala Val Gly Leu Ser Arg Ser Glu Val Gly
        675                 680                 685

Ile Tyr Ile Arg Gln Val Pro Leu Gln Ser Glu Glu Cys Leu Val Val
    690                 695                 700

Gly Cys Val Asn Ser Pro Ser Ser Val Thr Val Ser Gly Asp Leu Ser
705                 710                 715                 720

Ala Ile Ala Lys Leu Glu Glu Leu Leu His Ala Asp Arg Ile Phe Ala
                725                 730                 735

Arg Arg Leu Lys Val Thr Gln Ala Phe His Ser Ser His Met Asn Ser
            740                 745                 750

Met Thr Asp Ala Phe Arg Ala Gly Leu Thr Glu Leu Phe Gly Ala Asp
        755                 760                 765

Pro Ser Asp Ala Ala Asn Ala Ser Lys Asp Val Ile Tyr Ala Ser Pro
    770                 775                 780

Arg Thr Gly Ala Arg Leu His Asp Met Asn Arg Leu Arg Asp Pro Ile
785                 790                 795                 800

His Trp Val Glu Cys Met Leu His Pro Val Glu Phe Glu Ser Ala Phe
                805                 810                 815

Arg Arg Met Cys Leu Asp Glu Asn Asp His Met Pro Lys Val Asp Arg
            820                 825                 830

Val Ile Glu Ile Gly Pro His Gly Ala Leu Gly Gly Pro Ile Lys Gln
        835                 840                 845

Ile Met Gln Leu Pro Glu Leu Ala Thr Cys Asp Ile Pro Tyr Leu Ser
    850                 855                 860

Cys Leu Ser Arg Gly Lys Ser Ser Leu Ser Thr Leu Arg Leu Leu Ala
865                 870                 875                 880

Ser Glu Leu Ile Arg Ala Gly Phe Pro Val Asp Leu Asn Ala Ile Asn
                885                 890                 895
```



```
Phe Pro Arg Gly Cys Glu Ala Ala Arg Val Gln Val Leu Ser Asp Leu
            900                 905                 910

Pro Pro Tyr Pro Trp Asn His Glu Thr Arg Tyr Trp Lys Glu Pro Arg
        915                 920                 925

Ile Ser Gln Ser Ala Arg Gln Arg Lys Gly Pro Val His Asp Leu Ile
    930                 935                 940

Gly Leu Gln Glu Pro Leu Asn Leu Pro Leu Ala Arg Ser Trp His Asn
945                 950                 955                 960

Val Leu Arg Val Ser Asp Leu Pro Trp Leu Arg Asp His Val Val Gly
                965                 970                 975

Ser His Ile Val Phe Pro Gly Ala Gly Phe Val Cys Met Ala Val Met
            980                 985                 990

Gly Ile Ser Thr Leu Cys Ser Ser Asp His Glu Ser Asp Asp Ile Ser
        995                1000                1005

Tyr Ile Leu Arg Asp Val Asn Phe Ala Gln Ala Leu Ile Leu Pro Ala
   1010                1015                1020

Asp Gly Glu Glu Gly Ile Asp Leu Arg Leu Thr Ile Cys Ala Pro Asp
1025               1030                1035                1040

Gln Ser Leu Gly Ser Gln Asp Trp Gln Arg Phe Leu Val His Ser Ile
               1045                1050                1055

Thr Ala Asp Lys Asn Asp Trp Thr Glu His Cys Thr Gly Leu Val Arg
           1060                1065                1070

Ala Glu Met Asp Gln Pro Pro Ser Ser Leu Ser Asn Gln Gln Arg Ile
       1075                1080                1085

Asp Pro Arg Pro Trp Ser Arg Lys Thr Ala Pro Gln Glu Leu Trp Asp
   1090                1095                1100

Ser Leu His Arg Val Gly Ile Arg His Gly Pro Phe Phe Arg Asn Ile
1105               1110                1115                1120

Thr Cys Ile Glu Ser Asp Gly Arg Gly Ser Trp Cys Thr Phe Ala Ile
               1125                1130                1135

Ala Asp Thr Ala Ser Ala Met Pro His Ala Tyr Glu Ser Gln His Ile
           1140                1145                1150

Val His Pro Thr Thr Leu Asp Ser Ala Val Gln Ala Ala Tyr Thr Thr
       1155                1160                1165

Leu Pro Phe Ala Gly Ser Arg Ile Lys Ser Ala Met Val Pro Ala Arg
   1170                1175                1180

Val Gly Cys Met Lys Ile Ser Ser Arg Leu Ala Asp Leu Glu Ala Arg
1185               1190                1195                1200

Asp Met Leu Arg Ala Gln Ala Lys Met His Ser Gln Ser Pro Ser Ala
               1205                1210                1215

Leu Val Thr Asp Val Ala Val Phe Asp Glu Ala Asp Pro Val Gly Gly
           1220                1225                1230

Pro Val Met Glu Leu Glu Gly Leu Val Phe Gln Ser Leu Gly Ala Ser
       1235                1240                1245
```

Leu Gly Thr Ser Asp Arg Asp Ser Thr Asp Pro Gly Asn Thr Cys Ser
1250 1255 1260

Ser Trp His Trp Ala Pro Asp Ile Ser Leu Val Asn Pro Gly Trp Leu
1265 1270 1275 1280

Glu Lys Thr Leu Gly Thr Gly Ile Gln Glu His Glu Ile Ser Leu Ile
1285 1290 1295

Leu Glu Leu Arg Arg Cys Ser Val His Phe Ile Gln Glu Ala Met Glu
1300 1305 1310

Ser Leu Ser Val Gly Asp Val Glu Arg Leu Ser Gly His Leu Ala Lys
1315 1320 1325

Phe Tyr Ala Trp Met Gln Lys Gln Leu Ala Cys Ala Gln Asn Gly Glu
1330 1335 1340

Leu Gly Pro Glu Ser Ser Ser Trp Thr Arg Asp Ser Glu Gln Ala Arg
1345 1350 1355 1360

Cys Ser Leu Arg Ser Arg Val Val Ala Gly Ser Thr Asn Gly Glu Met
1365 1370 1375

Ile Cys Arg Leu Gly Ser Val Leu Pro Ala Ile Leu Arg Arg Glu Val
1380 1385 1390

Asp Pro Leu Glu Val Met Met Asp Gly His Leu Leu Ser Arg Tyr Tyr
1395 1400 1405

Val Asp Ala Leu Lys Trp Ser Arg Ser Asn Ala Gln Ala Ser Glu Leu
1410 1415 1420

Val Arg Leu Cys Cys His Lys Asn Pro Arg Ala Arg Ile Leu Glu Ile
1425 1430 1435 1440

Gly Gly Gly Thr Gly Gly Cys Thr Gln Leu Val Val Asp Ser Leu Gly
1445 1450 1455

Pro Asn Pro Pro Val Gly Arg Tyr Asp Phe Thr Asp Val Ser Ala Gly
1460 1465 1470

Phe Phe Glu Ala Ala Arg Lys Arg Phe Ala Gly Trp Gln Asn Val Met
1475 1480 1485

Asp Phe Arg Lys Leu Asp Ile Glu Asp Asp Pro Glu Ala Gln Gly Phe
1490 1495 1500

Val Cys Gly Ser Tyr Asp Val Val Leu Ala Cys Gln Val Leu His Ala
1505 1510 1515 1520

Thr Ser Asn Met Gln Arg Thr Leu Thr Asn Val Arg Lys Leu Leu Lys
1525 1530 1535

Pro Gly Gly Lys Leu Ile Leu Val Glu Thr Thr Arg Asp Glu Leu Asp
1540 1545 1550

Leu Phe Phe Thr Phe Gly Leu Leu Pro Gly Trp Trp Leu Ser Glu Glu
1555 1560 1565

Pro Glu Arg Gln Ser Thr Pro Ser Leu Ser Pro Thr Met Trp Arg Ser
1570 1575 1580

Met Leu His Thr Thr Gly Phe Asn Gly Val Glu Val Glu Ala Arg Asp
1585 1590 1595 1600

Cys Asp Ser His Glu Phe Tyr Met Ile Ser Thr Met Met Ser Thr Ala
1605 1610 1615

Val Gln Ala Thr Pro Met Ser Cys Ser Val Lys Leu Pro Glu Val Leu
1620 1625 1630

Leu Val Tyr Val Asp Ser Ser Thr Pro Met Ser Trp Ile Ser Asp Leu
1635 1640 1645

Gln Gly Glu Ile Arg Gly Arg Asn Cys Ser Val Thr Ser Leu Gln Ala
1650 1655 1660

Leu Arg Gln Val Pro Pro Thr Glu Gly Gln Ile Cys Val Phe Leu Gly
1665 1670 1675 1680

Glu Val Glu His Ser Met Leu Gly Ser Val Thr Asn Asp Asp Phe Thr
1685 1690 1695

Leu Leu Thr Ser Met Leu Gln Leu Ala Gly Gly Thr Leu Trp Val Thr
1700 1705 1710

Gln Gly Ala Thr Met Lys Ser Asp Asp Pro Leu Lys Ala Leu His Leu
1715 1720 1725

Gly Leu Leu Arg Thr Met Arg Asn Glu Ser His Gly Lys Arg Phe Val
1730 1735 1740

Ser Leu Asp Leu Asp Pro Ser Arg Asn Pro Trp Thr Gly Asp Ser Arg
1745 1750 1755 1760

Asp Ala Ile Val Ser Val Leu Asp Leu Ile Ser Met Ser Asp Glu Lys
1765 1770 1775

Glu Phe Asp Tyr Ala Glu Arg Asp Gly Val Ile His Val Pro Arg Ala
1780 1785 1790

Phe Ser Asp Ser Ile Asn Gly Gly Glu Glu Asp Gly Tyr Ala Leu Glu
1795 1800 1805

Pro Phe Gln Asp Ser Gln His Leu Leu Arg Leu Asp Ile Gln Thr Pro
1810 1815 1820

Gly Leu Leu Asp Ser Leu His Phe Thr Lys Arg Asn Val Asp Thr Tyr
1825 1830 1835 1840

Glu Pro Asp Lys Leu Pro Asp Asp Trp Val Glu Ile Glu Pro Arg Ala
1845 1850 1855

Phe Gly Leu Asn Phe Arg Asp Ile Met Val Ala Met Gly Gln Leu Glu
1860 1865 1870

Ser Asn Val Met Gly Phe Glu Cys Ala Gly Val Val Thr Ser Leu Ser
1875 1880 1885

Glu Thr Ala Arg Thr Ile Ala Pro Gly Leu Ala Val Gly Asp Arg Val
1890 1895 1900

Cys Ala Leu Met Asn Gly His Trp Ala Ser Arg Val Thr Thr Ser Arg
1905 1910 1915 1920

Thr Asn Val Val Arg Ile Pro Glu Thr Leu Ser Phe Pro His Ala Ala
1925 1930 1935

Ser Ile Pro Leu Ala Phe Thr Thr Ala Tyr Ile Ser Leu Tyr Thr Val
1940 1945 1950

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Ala Arg Ile Leu Pro Gly Glu Thr Val Leu Ile His Ala Gly Ala Gly
        1955                1960                1965

Gly Val Gly Gln Ala Ala Ile Ile Leu Ala Gln Leu Thr Gly Ala Glu
    1970                1975                1980

Val Phe Thr Thr Ala Gly Ser Glu Thr Lys Arg Asn Leu Leu Ile Asp
1985                1990                1995                2000

Lys Phe His Leu Asp Pro Asp His Val Phe Ser Ser Arg Asp Ser Ser
                2005                2010                2015

Phe Val Asp Gly Ile Lys Thr Arg Thr Arg Gly Lys Gly Val Asp Val
            2020                2025                2030

Val Leu Asn Ser Leu Ala Gly Pro Leu Leu Gln Lys Ser Phe Asp Cys
        2035                2040                2045

Leu Ala Arg Phe Gly Arg Phe Val Glu Ile Gly Lys Lys Asp Leu Glu
    2050                2055                2060

Gln Asn Ser Arg Leu Asp Met Ser Thr Phe Val Arg Asn Val Ser Phe
2065                2070                2075                2080

Ser Ser Val Asp Ile Leu Tyr Trp Gln Gln Ala Lys Pro Ala Glu Ile
                2085                2090                2095

Phe Gln Ala Met Ser Glu Val Ile Leu Leu Trp Glu Arg Thr Ala Ile
            2100                2105                2110

Gly Leu Ile His Pro Ile Ser Glu Tyr Pro Met Ser Ala Leu Glu Lys
        2115                2120                2125

Ala Phe Arg Thr Met Gln Ser Gly Gln His Val Gly Lys Ile Val Val
    2130                2135                2140

Thr Val Ala Pro Asp Asp Ala Val Leu Val Arg Gln Glu Arg Met Pro
2145                2150                2155                2160

Leu Phe Leu Lys Pro Asn Val Ser Tyr Leu Val Ala Gly Gly Leu Gly
                2165                2170                2175

Gly Ile Gly Arg Arg Ile Cys Glu Trp Leu Val Asp Arg Gly Ala Arg
            2180                2185                2190

Tyr Leu Ile Ile Leu Ser Arg Thr Ala Arg Val Asp Pro Val Val Thr
        2195                2200                2205

Ser Leu Gln Glu Arg Gly Cys Thr Val Ser Val Gln Ala Cys Asp Val
    2210                2215                2220

Ala Asp Glu Ser Gln Leu Glu Ala Ala Leu Gln Gln Cys Arg Ala Glu
2225                2230                2235                2240

Glu Met Pro Pro Ile Arg Gly Val Ile Gln Gly Ala Met Val Leu Lys
                2245                2250                2255

Asp Ala Leu Val Ser Gln Met Thr Ala Asp Gly Phe His Ala Ala Leu
            2260                2265                2270

Arg Pro Lys Val Gln Gly Ser Trp Asn Leu His Arg Ile Ala Ser Asp
        2275                2280                2285

Val Asp Phe Phe Val Met Leu Ser Ser Leu Val Gly Val Met Gly Gly
    2290                2295                2300
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Ala Gly Gln Ala Asn Tyr Ala Ala Ala Gly Ala Phe Gln Asp Ala Leu
2305                2310                2315                2320

Ala Glu His Arg Met Ala His Asn Gln Pro Ala Val Thr Ile Asp Leu
                2325                2330                2335

Gly Met Val Gln Ser Ile Gly Tyr Val Ala Glu Thr Asp Ser Ala Val
            2340                2345                2350

Ala Glu Arg Leu Gln Arg Ile Gly Tyr Gln Pro Leu His Glu Glu Glu
        2355                2360                2365

Val Leu Asp Val Leu Glu Gln Ala Ile Ser Pro Val Cys Ser Pro Ala
    2370                2375                2380

Ala Pro Thr Arg Pro Ala Val Ile Val Thr Gly Ile Asn Thr Arg Pro
2385                2390                2395                2400

Gly Pro His Trp Ala His Ala Asp Trp Met Gln Glu Ala Arg Phe Ala
                2405                2410                2415

Gly Ile Lys Tyr Arg Asp Pro Leu Arg Asp Asn His Gly Ala Leu Ser
            2420                2425                2430

Leu Thr Pro Ala Glu Asp Asp Asn Leu His Ala Arg Leu Asn Arg Ala
        2435                2440                2445

Ile Ser Gln Gln Glu Ser Ile Ala Val Ile Met Glu Ala Met Ser Cys
    2450                2455                2460

Lys Leu Ile Ser Met Phe Gly Leu Thr Asp Ser Glu Met Ser Ala Thr
2465                2470                2475                2480

Gln Thr Leu Ala Gly Ile Gly Val Asp Ser Leu Val Ala Ile Glu Leu
                2485                2490                2495

Arg Asn Trp Ile Thr Ala Lys Phe Asn Val Asp Ile Ser Val Phe Glu
            2500                2505                2510

Leu Met Glu Gly Arg Thr Ile Ala Lys Val Ala Glu Val Val Leu Gln
        2515                2520                2525

Arg Tyr Lys Ala
    2530


<210> 11
<211> 249
<212> PRT
<213> Aspergillus terreus

<400> 11
Met Ala Thr Gln Glu Phe Leu Ser Asp Val Ser Ser Gly Phe Leu Ser
1               5                   10                  15

Ala Glu Ala Ile Arg Tyr Arg Val Lys Thr Gly Val Ser Met Asp Gly
            20                  25                  30

Trp Met Lys Arg Gly Tyr Ser Cys Asn Ser Val Arg Thr Asp Asp Lys
        35                  40                  45

His His Leu Arg His Leu Thr Asn Ile Gly Leu Asp Thr Pro Pro Cys
    50                  55                  60

Pro Lys Ser Leu Pro Ala Ala His Ser Ala Val Ala Ser Cys Leu Thr
65                  70                  75                  80
```

Phe Val Pro Pro Asp Pro Cys Glu Asn Trp Glu Ala Leu Gln Val Ala
85 90 95

Trp Asp Lys Ala Cys Cys Arg Asn Pro Thr Pro Leu Phe Phe Ile Cys
100 105 110

Val Ser Leu Leu Phe Ser Phe Tyr Ser Leu Trp Leu Gln Arg Gly Gly
115 120 125

Cys Gly Arg Tyr Gly Gly Leu His Arg Val Ser Lys Val Phe Pro Lys
130 135 140

Val Trp Pro Asp Asp Met Asp Ser Gln Leu Pro Ser Arg Leu Gln Thr
145 150 155 160

Leu Val Ser Lys Arg Lys Pro Glu Pro Ala Pro Asn Asn Ser Thr Tyr
165 170 175

Ile Ser Lys Gly Tyr Ala Thr Phe Phe Asn Gln Phe Ser Leu Pro Ser
180 185 190

Val Asp Val Thr Gln Ile Leu Asn Gln Thr Leu Gln His His Asp Val
195 200 205

Glu Thr Ile Asn Leu Asp Cys Gly Ser Gly Leu Leu Thr Leu Arg Thr
210 215 220

Gln Leu Arg Ile Leu Leu Ile Gly Lys Pro Lys Ile Ile Lys Pro Phe
225 230 235 240

Ser Gly Leu Arg Thr Ser Ile Asn Glu
245

<210> 12
<211> 742
<212> PRT
<213> Aspergillus terreus

<400> 12
Met Glu Ser Ala Glu Leu Ser Ser Lys Arg Gln Ala Phe Pro Ala Cys
1 5 10 15

Asp Glu Cys Arg Ile Arg Lys Val Arg Cys Ser Lys Glu Gly Pro Lys
20 25 30

Cys Ser His Cys Leu Arg Tyr Asn Leu Pro Cys Glu Phe Ser Asn Lys
35 40 45

Val Ala Arg Asp Val Glu Lys Leu Gly Ser Arg Val Gly Asp Ile Glu
50 55 60

His Ala Leu Gln Arg Cys Leu Ser Phe Ile Asp Ala His Gln Gly Phe
65 70 75 80

Arg Asp Leu Ser Arg Pro Gln Ser Gln Glu Ser Gly Tyr Thr Ser Ser
85 90 95

Thr Ser Ser Glu Glu Cys Glu Val Asn Leu Tyr Ser Gly Lys His Thr
100 105 110

Ser Pro Thr Glu Glu Asp Gly Phe Trp Pro Leu His Gly Tyr Gly Ser
115 120 125

Phe Val Ser Leu Val Met Glu Ala Gln Ala Ala Asn Ala Asn Leu Thr
130 135 140

Ser Trp Leu Pro Val Asp Met Thr Ser Gly Gln Val Ala Glu Met Val
145 150 155 160

Ala Phe Asp Arg Gln Ala Val Ser Ala Val Arg Ser Lys Val Ala Glu
165 170 175

Ala Asn Glu Thr Leu Gln Gln Ile Ile Glu Asp Ile Pro Thr Leu Ser
180 185 190

Ala Ser Glu Asn Asp Thr Phe Leu Pro Ser Leu Pro Pro Arg Ala Leu
195 200 205

Val Glu Pro Ser Ile Asn Glu Tyr Phe Lys Lys Leu His Pro Arg Leu
210 215 220

Pro Ile Phe Ser Arg Gln Thr Ile Met Asp Ala Val Glu Ser Gln Tyr
225 230 235 240

Thr Ile Arg Thr Gly Pro Pro Asp Leu Val Trp Ile Thr Ser Phe Asn
245 250 255

Cys Ile Val Leu Gln Ala Leu Thr Gln Thr Ser Ile Ala Asn Lys Val
260 265 270

Val Gly Cys Thr Gly Gln Asp Ile Pro Ile Asp Tyr Met Ile Ile Ser
275 280 285

Leu Leu Arg Asn Ile Arg Gln Cys Tyr Asn Arg Leu Glu Thr Leu Val
290 295 300

Lys Pro Arg Leu Ser Asn Ile Arg Ala Leu Phe Cys Leu Ala Leu Val
305 310 315 320

Ala Met Glu Tyr Phe Asp Phe Ala Ile Phe Leu Thr Ile Phe Ala Gln
325 330 335

Val Cys Glu Leu Ser Arg Leu Ile Gly Leu His Leu Thr Thr Thr Thr
340 345 350

Pro Pro Thr Glu Asp Gly Ala Val Gly Asp Gln Pro Lys Asp Leu Phe
355 360 365

Trp Ser Ile Phe Leu Val Asp Lys His Val Ser Ile Ile Gly Gly Lys
370 375 380

Ala Cys Leu Leu Pro Ser Tyr Asp Cys Ser Val Pro Leu Pro Pro Tyr
385 390 395 400

Asp Ser Ala Ala Pro Leu Pro Asn Ala Phe Ala Ala Arg Ile Arg Leu
405 410 415

Ala Phe Ile Leu Glu Glu Ile Tyr Leu Gly Leu Tyr Ser Ala Lys Ser
420 425 430

Ser Lys Met Glu Gln Ser Arg Val Arg Arg Arg Ile Arg Arg Ile Ala
435 440 445

Arg Lys Leu Ser Gln Trp His Val Gln His Glu His Val Leu Arg Thr
450 455 460

Gly Asp Pro Asn Arg Pro Leu Glu Glu Tyr Ile Cys Ala Thr Gln Leu
465 470 475 480

Arg Phe Ala Leu Ser Ser Cys Trp Val Leu Leu His Lys Arg Ile Trp
485 490 495

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Ser Gln Glu Arg Gly Ala Val Cys Leu Gln His Ala Arg Asp Cys Leu
            500                 505                 510

Met Leu Phe Lys Gln Leu Cys Asp Gly Cys Lys Ser Gly Phe Ser Asn
        515                 520                 525

Phe Asp Ser Ile Val Leu Asn Tyr Ser Leu Ile Ser Phe Met Gly Ile
    530                 535                 540

Tyr Val His Ile Val Glu Glu Asp Gln Pro Ile His Ser Gln Asp Met
545                 550                 555                 560

Glu Ile Leu Thr Phe Phe Ala Ile Tyr Thr Asn Arg Ser Ala Ser Asn
                565                 570                 575

Arg Ser Ser Ala Ser Ile Ser Tyr Lys Leu Ser Gln Val Ala Ser Arg
            580                 585                 590

Cys Ser Asp Ile Ala Leu Leu Leu Gln Asn Leu Arg Glu Arg Arg Phe
        595                 600                 605

Ile Pro Thr Thr Ile Ser Arg Ser Pro Thr Pro Ser Trp Asn Glu Pro
    610                 615                 620

Thr Tyr Met Asp Tyr Asp Val Ala Asn Ala Ser Thr Ser Thr Thr Ser
625                 630                 635                 640

Thr Gly Ser Ser Tyr Asn Leu Asn Ile Ser Pro Leu Gly Val Pro Gly
                645                 650                 655

Asp Gly Gln Val Trp Asp Ile Tyr Phe Asn Pro Arg Glu Ile Pro Met
            660                 665                 670

Asp Gly Thr Ile Ala Thr Pro Ser Glu Asp Ala Thr Gln Asp Leu Leu
        675                 680                 685

Ser Asn Asp Ala Gly Gln Cys Leu Gly Phe Pro Asp Phe Ser Leu Gly
    690                 695                 700

Ile Asp Asn Phe Ser Asp Phe Pro Leu Gly Ile Asp Met Thr Ser Gln
705                 710                 715                 720

Ser Glu Phe Gly Leu Ile Met Glu Glu Asp Ile Ile Arg Tyr Glu Arg
                725                 730                 735

Leu Leu Asp Arg Pro Val
            740


<210> 13
<211> 301
<212> PRT
<213> Aspergillus terreus

<400> 13
Met Glu Ser Lys Val Gln Thr Asn Val Pro Leu Pro Lys Ala Pro Leu
1               5                   10                  15

Thr Gln Lys Ala Arg Gly Lys Arg Thr Lys Gly Ile Pro Ala Leu Val
            20                  25                  30

Ala Gly Ala Cys Ala Gly Ala Val Glu Ile Ser Ile Thr Tyr Pro Phe
        35                  40                  45

Glu Ser Ala Lys Thr Arg Ala Gln Leu Lys Arg Arg Asn His Asp Val
    50                  55                  60
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Ala Ala Ile Lys Pro Gly Ile Arg Gly Trp Tyr Ala Gly Tyr Gly Ala
65                  70                  75                  80

Thr Leu Val Gly Thr Thr Leu Lys Ala Ser Val Gln Phe Ala Ser Phe
                85                  90                  95

Asn Ile Tyr Arg Ser Ala Leu Ser Gly Pro Asn Gly Glu Leu Ser Thr
            100                 105                 110

Gly Ala Ser Val Leu Ala Gly Phe Gly Ala Gly Val Thr Glu Ala Val
        115                 120                 125

Leu Ala Val Thr Pro Ala Glu Ala Ile Lys Thr Lys Ile Ile Asp Ala
    130                 135                 140

Arg Lys Val Gly Asn Ala Glu Leu Ser Thr Thr Phe Gly Ala Ile Ala
145                 150                 155                 160

Gly Ile Leu Arg Asp Arg Gly Pro Leu Gly Phe Phe Ser Ala Val Gly
                165                 170                 175

Pro Thr Ile Leu Arg Gln Ser Ser Asn Ala Ala Val Lys Phe Thr Val
            180                 185                 190

Tyr Asn Glu Leu Ile Gly Leu Ala Arg Lys Tyr Ser Lys Asn Gly Glu
        195                 200                 205

Asp Val His Pro Leu Ala Ser Thr Leu Val Gly Ser Val Thr Gly Val
    210                 215                 220

Cys Cys Ala Trp Ser Thr Gln Pro Leu Asp Val Ile Lys Thr Arg Met
225                 230                 235                 240

Gln Ser Leu Gln Ala Arg Gln Leu Tyr Gly Asn Thr Phe Asn Cys Val
                245                 250                 255

Lys Thr Leu Leu Arg Asn Glu Gly Ile Gly Val Phe Trp Ser Gly Val
            260                 265                 270

Trp Phe Arg Thr Gly Arg Leu Ser Leu Thr Ser Ala Ile Met Phe Pro
        275                 280                 285

Val Tyr Glu Lys Val Tyr Lys Phe Leu Thr Gln Pro Asn
    290                 295                 300


<210> 14
<211> 490
<212> PRT
<213> Aspergillus terreus

<400> 14
Met Thr Lys Gln Ser Ala Asp Ser Asn Ala Lys Ser Gly Val Thr Ala
1               5                   10                  15

Glu Ile Cys His Trp Ala Ser Asn Leu Ala Thr Asp Asp Ile Pro Pro
            20                  25                  30

Asp Val Leu Glu Arg Ala Lys Tyr Leu Ile Leu Asp Gly Ile Ala Cys
        35                  40                  45

Ala Trp Val Gly Ala Arg Val Pro Trp Ser Glu Lys Tyr Val Gln Ala
    50                  55                  60

Thr Met Ser Phe Glu Pro Pro Gly Ala Cys Arg Val Ile Gly Tyr Gly
65                  70                  75                  80
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Gln Lys Leu Gly Pro Val Ala Ala Ala Met Thr Asn Ser Ala Phe Ile
85 90 95

Gln Ala Thr Glu Leu Asp Asp Tyr His Ser Glu Ala Pro Leu His Ser
100 105 110

Ala Ser Ile Val Leu Pro Ala Val Phe Ala Ala Ser Glu Val Leu Ala
115 120 125

Glu Gln Gly Lys Thr Ile Ser Gly Ile Ala Val Ile Leu Ala Ala Ile
130 135 140

Val Gly Phe Glu Ser Gly Pro Arg Ile Gly Lys Ala Ile Tyr Gly Ser
145 150 155 160

Asp Leu Leu Asn Asn Gly Trp His Cys Gly Ala Val Tyr Gly Ala Pro
165 170 175

Ala Gly Ala Leu Ala Thr Gly Lys Leu Leu Gly Leu Thr Pro Asp Ser
180 185 190

Met Glu Asp Ala Leu Gly Ile Ala Cys Thr Gln Ala Cys Gly Leu Met
195 200 205

Ser Ala Gln Tyr Gly Gly Met Val Lys Arg Val Gln His Gly Phe Ala
210 215 220

Ala Arg Asn Gly Leu Leu Gly Gly Leu Leu Ala His Gly Gly Tyr Glu
225 230 235 240

Ala Met Lys Gly Val Leu Glu Arg Ser Tyr Gly Gly Phe Leu Lys Met
245 250 255

Phe Thr Lys Gly Asn Gly Arg Glu Pro Pro Tyr Lys Glu Glu Glu Val
260 265 270

Val Ala Gly Leu Gly Ser Phe Trp His Thr Phe Thr Ile Arg Ile Lys
275 280 285

Leu Tyr Ala Cys Cys Gly Leu Val His Gly Pro Val Glu Ala Ile Glu
290 295 300

Asn Leu Gln Arg Arg Tyr Pro Glu Leu Leu Asn Arg Ala Asn Leu Ser
305 310 315 320

Asn Ile Arg His Val His Val Gln Leu Ser Thr Ala Ser Asn Ser His
325 330 335

Cys Gly Trp Ile Pro Glu Glu Arg Pro Ile Ser Ser Ile Ala Gly Gln
340 345 350

Met Ser Val Ala Tyr Ile Leu Ala Val Gln Leu Val Asp Gln Gln Cys
355 360 365

Leu Leu Ala Gln Phe Ser Glu Phe Asp Asp Asn Leu Glu Arg Pro Glu
370 375 380

Val Trp Asp Leu Ala Arg Lys Val Thr Pro Ser His Ser Glu Glu Phe
385 390 395 400

Asp Gln Asp Gly Asn Cys Leu Ser Ala Gly Arg Val Arg Ile Glu Phe
405 410 415

Asn Asp Gly Ser Ser Val Thr Glu Thr Val Glu Lys Pro Leu Gly Val
420 425 430

Lys Glu Pro Met Pro Asn Glu Arg Ile Leu His Lys Tyr Arg Thr Leu
435 440 445

Ala Gly Ser Val Thr Asp Glu Thr Arg Val Lys Glu Ile Glu Asp Leu
450 455 460

Val Leu Ser Leu Asp Arg Leu Thr Asp Ile Ser Pro Leu Leu Glu Leu
465 470 475 480

Leu Asn Cys Pro Val Lys Ser Pro Leu Val
485 490

<210> 15
<211> 488
<212> PRT
<213> Aspergillus terreus

<400> 15
Met Gly Arg Gly Asp Thr Glu Ser Pro Asn Pro Ala Thr Thr Ser Glu
1 5 10 15

Gly Ser Gly Gln Asn Glu Pro Glu Lys Lys Gly Arg Asp Ile Pro Leu
20 25 30

Trp Arg Lys Cys Val Ile Thr Phe Val Val Ser Trp Met Thr Leu Val
35 40 45

Val Thr Phe Ser Ser Thr Cys Leu Leu Pro Ala Ala Pro Glu Ile Ala
50 55 60

Asn Glu Phe Asp Met Thr Val Glu Thr Ile Asn Ile Ser Asn Ala Gly
65 70 75 80

Val Leu Val Ala Met Gly Tyr Ser Ser Leu Ile Trp Gly Pro Met Asn
85 90 95

Lys Leu Val Gly Arg Arg Thr Ser Tyr Asn Leu Ala Ile Ser Met Leu
100 105 110

Cys Ala Cys Ser Ala Gly Thr Ala Ala Ala Ile Asn Glu Lys Met Phe
115 120 125

Ile Ala Phe Arg Val Leu Ser Gly Leu Thr Gly Thr Ser Phe Met Val
130 135 140

Ser Gly Gln Thr Val Leu Ala Asp Ile Phe Glu Pro Val Tyr Arg Gly
145 150 155 160

Thr Ala Val Gly Phe Phe Met Ala Gly Thr Leu Ser Gly Pro Ala Ile
165 170 175

Gly Pro Cys Val Gly Gly Val Ile Val Thr Phe Thr Ser Trp Arg Val
180 185 190

Ile Phe Trp Leu Gln Leu Gly Met Ser Gly Leu Gly Leu Val Leu Ser
195 200 205

Leu Leu Phe Phe Pro Lys Ile Glu Gly Thr Ser Glu Lys Val Ser Thr
210 215 220

Ala Phe Lys Pro Thr Thr Leu Val Ser Ile Ile Ser Lys Phe Ser Pro
225 230 235 240

Thr Asp Val Leu Lys Gln Trp Val Tyr Pro Asn Val Phe Leu Ala Val
245 250 255

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Ser Ala Trp Glu Ile Cys Pro Leu His Leu Leu Glu Thr Lys Cys Ser
            260                 265                 270

Cys Arg Lys Gln Lys Asp Leu Cys Cys Gly Leu Leu Ala Ile Thr Gln
        275                 280                 285

Tyr Ser Ile Leu Thr Ser Ala Arg Ala Ile Phe Asn Ser Arg Phe His
    290                 295                 300

Leu Thr Thr Ala Leu Val Ser Gly Leu Phe Tyr Leu Ala Pro Gly Ala
305                 310                 315                 320

Gly Phe Leu Ile Gly Ser Leu Val Gly Gly Lys Leu Ser Asp Arg Thr
                325                 330                 335

Val Arg Arg Tyr Ile Val Lys Arg Gly Phe Arg Leu Pro Gln Asp Arg
            340                 345                 350

Leu His Ser Gly Leu Ile Thr Leu Phe Ala Val Leu Pro Ala Gly Thr
        355                 360                 365

Leu Ile Tyr Gly Trp Thr Leu Gln Glu Asp Lys Gly Gly Met Val Val
    370                 375                 380

Pro Ile Ile Ala Ala Phe Phe Ala Gly Trp Gly Leu Met Gly Ser Phe
385                 390                 395                 400

Asn Cys Leu Asn Thr Tyr Val Ala Val Glu Ala Leu Pro Arg Asn Arg
                405                 410                 415

Ser Ala Val Ile Ala Gly Lys Tyr Met Ile Gln Tyr Ser Phe Ser Ala
            420                 425                 430

Gly Ser Ser Ala Leu Val Val Pro Val Ile Asp Ala Leu Gly Val Gly
        435                 440                 445

Trp Thr Phe Thr Leu Cys Val Val Ala Ser Thr Ile Ala Gly Leu Ile
    450                 455                 460

Thr Ala Ala Ile Ala Arg Trp Gly Ile Asn Met Gln Arg Trp Ala Glu
465                 470                 475                 480

Arg Ala Phe Asn Leu Pro Thr Gln
                485


<210> 16
<211> 516
<212> PRT
<213> Aspergillus terreus

<400> 16
Met Thr Leu Gln Ile Ile Val Ile Ala Ala Thr Ala Val Ile Tyr Phe
 1               5                  10                  15

Leu Thr Arg Tyr Phe Asn Arg Thr Asp Ile Pro Lys Ile Lys Gly Ile
            20                  25                  30

Pro Glu Ile Pro Gly Val Pro Ile Phe Gly Asn Leu Ile Gln Leu Gly
        35                  40                  45

Val Lys His Ala Thr Val Ala Arg Lys Trp Ser Lys Glu Phe Gly Pro
    50                  55                  60

Val Phe Gln Ala Arg Leu Gly Asn Arg Arg Val Ile Phe Ala Asn Thr
 65                 70                  75                  80
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Phe Glu Ser Thr Arg Gln Leu Trp Ile Lys Glu Gln Ser Ser Met Ile
                85                  90                  95

Ser Arg Pro Thr Phe His Thr Phe His Gly Val Val Ser Ser Ser Gln
            100                 105                 110

Gly Phe Thr Ile Gly Thr Ser Pro Trp Asp Glu Ser Cys Lys Arg Arg
        115                 120                 125

Arg Lys Ala Ala Ala Thr Ala Leu Asn Arg Val Ala Val Gln Ser Tyr
    130                 135                 140

Met Pro Ile Ile Asp Leu Glu Ser Met Ala Ser Ile Lys Glu Leu Leu
145                 150                 155                 160

Lys Asp Ser Gln Gly Gly Lys Ile Asp Ile Asn Pro Thr Pro Tyr Phe
                165                 170                 175

Gln Arg Phe Ala Leu Asn Thr Ser Leu Thr Leu Asn Tyr Gly Tyr Arg
            180                 185                 190

Ile Glu Gly Asn Val Asn Asp Gln Leu Leu Arg Glu Ile Cys Glu Val
        195                 200                 205

Gln Arg Gly Val Ala Asn Leu Arg Ser Thr Ser Asn Asn Trp Gln Asp
    210                 215                 220

Tyr Val Pro Leu Leu Arg Leu Phe Ser Asn Arg Ser Asn Gln Ala Lys
225                 230                 235                 240

His Leu Arg Ala Arg Arg Asp Lys Tyr Met Ala Phe Leu Phe Asp Ile
                245                 250                 255

Leu Lys Asp Arg Met Ala Lys Gly Thr Asp Lys Pro Cys Ile Thr Gly
            260                 265                 270

Asn Ile Leu Lys Asn Pro Glu Thr Lys Leu Thr Asp Ala Glu Ile Lys
        275                 280                 285

Ser Ile Cys Leu Thr Met Val Ser Ala Gly Leu Asp Thr Val Pro Gly
    290                 295                 300

Asn Leu Ile Met Gly Ile Ala Tyr Leu Ser Ser Glu Asp Gly Gln Arg
305                 310                 315                 320

Ile Gln Gln Lys Ala Tyr Glu Glu Ile Met Ser Val Tyr Pro Asn Gly
                325                 330                 335

Asp Ala Trp Glu Arg Cys Leu Val Glu Glu Lys Val Pro Tyr Ile Thr
            340                 345                 350

Ala Leu Val Lys Glu Thr Leu Arg Phe Trp Thr Val Met Pro Ile Cys
        355                 360                 365

Ile Pro Arg Val Asn Ile Lys Glu Val Ile Tyr Asn Gly Ala Arg Ile
    370                 375                 380

Pro Ala Gly Thr Thr Phe Phe Met Asn Ala Trp Ala Ala Asn Tyr Asp
385                 390                 395                 400

Glu Asp His Phe Asp Met Pro Asn Arg Phe Leu Pro Glu Arg Tyr Leu
                405                 410                 415

Glu Pro Ser Glu Gly Phe Gly Thr Pro His Tyr Ser Phe Gly Ala Gly
            420                 425                 430
```

Thr Arg Met Cys Ala Ala Ser His Leu Ala Ser Arg Glu Leu Tyr Thr
435 440 445

Val Phe Leu Arg Phe Ile Val Ala Phe Thr Ile Glu Pro Ala Gln Asn
450 455 460

Pro Ala Asp Met Pro Val Leu Asp Ala Ile Glu Cys Asn Ala Thr Pro
465 470 475 480

Thr Ser Met Thr Thr Glu Pro Lys Pro Phe Lys Val Gly Phe Lys Pro
485 490 495

Arg Asp Glu Thr Ser Leu Arg Arg Trp Ile Ala Glu Ser Glu Glu Arg
500 505 510

Thr Lys Glu Leu
515

<210> 17
<211> 481
<212> PRT
<213> Aspergillus terreus

<400> 17
Met Lys Pro Ala Ile Leu Met Lys Tyr Trp Leu Phe Val Ser Ala Val
1 5 10 15

Ser Ala Ser Thr Leu Asn Gly Lys Leu Thr Leu Ser Glu Thr Lys Val
20 25 30

Thr Gly Ala Val Gln Leu Ala Cys Thr Asn Ser Pro Pro Asp Ile Tyr
35 40 45

Ile Asp Pro Asp Asp Ser Val Ser Val Val Arg Ala Ala His Asp Leu
50 55 60

Ala Leu Asp Phe Gly Arg Val Phe Gly Lys Asn Ala Thr Val Arg Phe
65 70 75 80

Thr Asn Glu Thr His Pro Thr Ser Met Ala Ile Ile Ala Gly Thr Ile
85 90 95

Asp Lys Ser Thr Phe Leu Gln Arg Leu Ile Ala Asp His Lys Leu Asp
100 105 110

Val Thr Ser Ile Arg Gly Gln Trp Glu Ser Tyr Ser Ser Ala Leu Val
115 120 125

Leu Gly Pro Ala Lys Gly Ile Gln Asn Ala Leu Val Ile Ala Gly Ser
130 135 140

Asp Arg Arg Gly Ala Ile Tyr Gly Leu Tyr Asp Ile Ser Glu Gln Ile
145 150 155 160

Gly Val Ser Pro Leu Phe Trp Trp Thr Asp Val Thr Pro Thr Lys Leu
165 170 175

Asp Ala Ile Tyr Ala Leu Asp Val Gln Lys Val Gln Gly Pro Pro Ser
180 185 190

Val Lys Tyr Arg Gly Ile Phe Ile Asn Asp Glu Ala Pro Ala Leu His
195 200 205

Asn Trp Ile Leu Ala Asn Tyr Gly Glu Val Glu Asn Gly Asp Pro Ala
210 215 220

Phe Ile Ser Arg Phe Tyr Ala His Val Phe Glu Leu Ile Leu Arg Leu
225 230 235 240

Lys Gly Asn Tyr Leu Trp Pro Ala Met Trp Ser Asn Met Phe Tyr Val
245 250 255

Asp Asp Thr Asn Asn Gly Pro Leu Ala Asp Tyr Tyr Gly Val Val Met
260 265 270

Gly Thr Ser His Thr Gly Met Thr Val Gly Thr Pro Cys Leu Lys Ala
275 280 285

His Ala Asp Tyr Glu Lys Glu Pro Met Ala Arg Ala Thr Asn Glu Gln
290 295 300

Ser Gln Phe Leu Asn Gly Thr Trp Asp Trp Ile Ser Asn Glu Val Asn
305 310 315 320

Val Lys Ala Phe Met Arg Glu Gly Val Ile Arg Ser Gln His Trp Glu
325 330 335

Thr Ala Tyr Thr Met Gly Met Arg Gly Leu Gly Asp Ala Ala Ser Pro
340 345 350

Thr Leu Asn Ala Thr Val Glu Glu Ser Ile Val Ser Trp Gln Glu Ser
355 360 365

Val Leu Ser Asp Ile Leu Asn Lys Thr Asn Leu Ser Asn Val Val Gln
370 375 380

Pro Phe Val Leu Phe Asp Glu Leu Gly Thr Tyr Tyr Glu Ser Gly Met
385 390 395 400

Thr Val Pro Asp Gln Val Thr Leu Ile Tyr Pro Asp Asp Asn Ala Gly
405 410 415

Asn Met Leu Arg Leu Pro Leu Gln Asn Glu Thr Gly Arg Ser Gly Gly
420 425 430

Ala Gly Ile Tyr Tyr His Phe Asp Met Asn Ala Pro Pro Arg Cys Tyr
435 440 445

Lys Trp Ile Asn Thr Ala Gln Leu Ile Arg Thr Trp Asp Gln Leu Arg
450 455 460

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